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Item: 3153

MICROBIAL PRODUCTS OF BIOTECHNOLOGY

FINAL REGULATION UNDER THE TOXIC SUBSTANCES CONTROL ACT

A SUMMARY OF THE PUBLIC'S COMMENTS AND THE AGENCY'S RESPONSE

US Environmental Protection Agency

March 26, 1997

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The Environmental Protection Agency (EPA) issued a proposal in the September 1, 1994 FEDERAL REGISTER to add to Title 40 of the Code of Federal Regulations (CFR) a Part 725, Reporting Requirements and Review Processes for Microorganisms. This proposal also revised 40 CFR Parts 700, 720, 721, and 723. The addition of part 725 would establish a screening system specifically designed for new microorganisms under TSCA § 5. The revisions to part 700 added fee requirements specifically for microorganism submissions under TSCA § 5. The proposed revisions to parts 720, 721, and 723 made reference to the new part 725 as containing the requirements for microorganisms subject to TSCA § 5.

In response to the proposal, EPA received 40 letters from the public during the comment period. The Microbial Products of Biotechnology Proposal docket (OPPTS-00049C) contains the proposal, materials supporting the proposal, public comments on the proposal, material EPA has added in reply to the public comments, and the Regulatory Impact Analyses for the proposed and the final rules.

After a careful review and analysis of the comments and data in the record, EPA is revising 40 CFR Parts 700, 720, 721, and 723, and adding part 725.

This document summarizes the public's comments on the proposed rule and presents EPA's response.

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I. GENERAL COMMENTS ON PROPOSED RULE

The proposed rule described implementation of EPA's program for regulation of microorganisms under TSCA § 5. In 1986 as part of the Office of Science and Technology Policy's "Coordinated Framework for Regulation of Biotechnology," EPA issued a policy statement which provided EPA's plans and rationale for addressing microbial products of biotechnology subject to TSCA ("1986 Policy Statement")(51 FR 23313, June 26, 1986). EPA was able to implement part of its TSCA microorganism program with the publication of the 1986 Policy Statement. However, to fully implement its TSCA program, additional rulemaking was necessary. The 1994 proposed rule was intended to provide the additional regulations needed to fully implement the TSCA § 5 program for microorganisms.

Since 1986, EPA has been reviewing notices on new microorganisms under TSCA § 5 using the procedures already in place for traditional chemicals. Under TSCA § 5, EPA must receive premanufacture notices (PMNs) prior to the introduction of new chemical substances into commerce. Microorganisms are considered "chemical substances" subject to TSCA. The 1994 proposed rule was intended to incorporate many of the procedures in the existing TSCA § 5 program for traditional chemicals with modifications, as appropriate, to address the specific characteristics of microorganisms. Additionally, the 1994 proposed rule contained exemptions for specific microorganisms under certain conditions of use based on EPA's experience reviewing notices for microorganisms under the 1986 Policy Statement.

EPA proposed to retain the 1986 Policy Statement interpretation of intergeneric microorganisms as "new microorganisms." Intergeneric microorganisms are those formed by combining genetic material from organisms in different taxonomic genera. The 1994 proposed rule also incorporated

the existing requirement that persons intending to manufacture (including import) or process new microorganisms for commercial purposes in the U.S. submit a notice to EPA at least 90 days before such manufacture or processing. EPA would have 90 days to review the submission to determine whether the intergeneric microorganism may present an unreasonable risk to human health or the environment. To distinguish between notices for traditional chemicals and microorganisms, the microorganism notice was renamed the Microbial Commercial Activity Notice or MCAN. The proposal also contained an exemption from MCAN requirements for certain listed microorganisms when they are produced in compliance with the specified exemption criteria.

At the research and development (R&D) stage, EPA proposed to exempt intergeneric microorganisms from TSCA § 5 reporting when they are tested in contained structures such as laboratories and greenhouses when certain requirements are met. In an effort to avoid duplicative Federal regulatory requirements, EPA also proposed to exempt from oversight those R&D activities which are conducted in contained structures and are subject to the joint jurisdiction of EPA and another Federal agency.

For commercial R&D activities involving intentional testing in the environment, the proposal included a requirement to submit a TSCA Experimental Release Application (TERA) to EPA at least 60 days before initiating such testing. EPA would have 60 days to review the submission and determine whether the proposed testing will not present an unreasonable risk to human health or the environment. This abbreviated reporting process was designed as an exemption from the full 90-day MCAN reporting process for microorganisms ready for commercialization. The proposal also contained an exemption from TERA reporting for certain listed microorganisms when they are tested in the environment under specific conditions.

Comments:

EPA received 40 comments on the proposed TSCA biotechnology rule. A list of the commenters and the corresponding numbers used for reference in this document may be found in Unit VI.A. One commenter was inadvertently given two separate numbers (#6 and 19; 19 is not used for reference). Three of the 40 comments (#1, 3, 10) were strictly requests for extensions of the rule comment period. EPA did extend the comment period for an extra 60 days beyond the original 60-day comment period. The other 36 commenters had raised issues about specific aspects of the rule. Nevertheless, 15 of the 36 commenters (#12, 15, 30, 8, 18, 22, 24, 35, 34, 25, 23, 26, 39, 32, 36) also indicated that they generally supported the rule. Eight of the 36 commenters (#13, 2, 5, 6, 14, 20, 9, 16) had major concerns about the rule and requested modifications that would significantly change the nature of the rule as proposed.

Of the 15 commenters expressing general support for the rule, six (#8, 18, 23, 25, 26, 30) specifically indicated that they believed it to be a significant improvement over the current system. Two commenters (#12, 25) were pleased that EPA had been responsive to their comments on

earlier drafts of the proposal which had been made available to the public. Three commenters (#8, 18, 25) expressed support for the "flexible and multistage process" which established a special R&D reporting program and other appropriate exemptions from full reporting. Two commenters (#12, 35) urged EPA to promulgate a final rule as quickly as possible.

Of the eight commenters expressing major concerns about the proposed rule, one (#13), while indicating support for a formal regulatory program, stated that "the Agency has not adequately justified the focus of the regulations solely upon microorganisms which are produced through genetic engineering processes" and recommended that "the Agency reevaluate its policy within the context of a more scientific risk-based approach to the regulation of microorganisms."

The other seven commenters (#2, 5, 6, 9, 14, 16, and 20) who raised major concerns about the proposal supported greater regulatory restrictions on intergeneric microorganisms, particularly those released to the environment. For example, these commenters opposed exemptions from review for any microorganisms prior to introduction into the environment (#2, 5, 6, 14, and 20), and objected to “blanket exemptions based on parent organism safety....” (#16).

EPA response:

EPA is promulgating final regulations which contain relatively few changes from the 1994 proposal. The specific changes and EPA's responses to commenters' recommendations are discussed in detail in the appropriate sections of this document. EPA believes that the proposed rule represents a significant improvement over the program it has been operating under the 1986 Policy Statement. These regulations are modelled after and incorporate many of the procedures in the existing TSCA § 5 program for traditional chemical substances that EPA has operated for the past decade, modified as appropriate to address the specific characteristics of microorganisms.

Section 5(h)(4) allows EPA to exempt new substances from all or part of § 5 reporting requirements, if EPA determines, by rule, that such substances will not present an unreasonable risk. EPA has used § 5(h)(4) to exempt certain categories of microorganisms from review as new microorganisms. These exemptions have been developed after careful review of the available scientific information and microorganism premanufacture notices (PMNs) submitted under TSCA § 5, as well as consideration of public comments and the advice of EPA's Biotechnology Science Advisory Committee (BSAC).

EPA believes that it has provided adequate justification for the program that it has developed. A diverse group of microorganisms could potentially be subject to TSCA. In general, EPA has created a flexible process which will allow for further refinement as EPA gains additional experience reviewing intergeneric microorganisms that are subject to TSCA § 5 oversight.

Many of the provisions of the final rule, and specifically the exemptions, are based on experience EPA has gained during its reviews of submissions for TSCA uses of intergeneric microorganisms

since 1986. EPA believes that the regulations it has developed for microorganisms conform with those imposed explicitly by TSCA § 5 but have been modified to accommodate issues specific to microorganisms. The exemptions are based on previous EPA decisions on reviewed microorganisms and existing industry practices. They permit activities with intergeneric microorganisms under conditions which EPA has already reviewed and approved.

More specific comments about issues in the proposed rule are addressed in the remainder of this document.

II. STATUTORY FRAMEWORK - COVERAGE UNDER TSCA

A. APPLICABILITY OF TSCA TO MICROORGANISMS

In the proposal EPA reiterated its interpretation, first stated in 1977, and discussed at length in 1984 (49 FR 50886, December 31, 1984), that microorganisms could be considered "chemical substances" subject to TSCA.

Comments:

EPA received six comments (#12, 13, 15, 18, 37, 40) on the applicability of TSCA to microorganisms. Three commenters (#12, 18, 40) indicated their support for TSCA coverage of microorganisms, and three commenters (#13, 15, 37) did not believe that it was appropriate to use TSCA to regulate microorganisms. One commenter (#13) noted that they:

do not believe that it is appropriate to justify TSCA's jurisdiction over microorganisms through adoption of a definition of chemical substances which proposes that because DNA, RNA and microorganisms in general are the result of combinations of "chemicals" and chemical reactions, they can be considered chemical substances under TSCA, thus establishing EPA's authority over them. While we acknowledge that EPA has operated under this policy since 1984, we believe this logic is also flawed, both scientifically and legally. In terms of science, DNA, RNA and living things in general are composed of biochemicals produced through biochemical reactions which are driven by enzymes.

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TSCA extended its authority to microorganisms, specifically those intergeneric microorganisms which are formed as a result of deliberate introduction of genetic material, in 1984 without justification as to the relationship of such microorganisms in terms of their effect on health and the environment, to the chemical substances which were the original focus of TSCA.

One commenter (#12) was concerned that EPA's authority to cover microorganisms under TSCA is ambiguous and suggested that the statute be amended to clearly apply to microorganisms. Another commenter (#15) expressed concern that the interpretation would be vulnerable to a

court challenge. This commenter suggested that a new comprehensive biotechnology regulatory statute was the appropriate alternative.

Two other commenters, while stating that they thought it was inappropriate to use TSCA to cover microorganisms, nevertheless suggested alternative approaches for covering microorganisms under TSCA. One of these commenters (#37) "question[ed] the appropriateness of the Toxic Substance Control Act to regulate recombinant microorganisms" and suggested that EPA consider developing a regulatory approach tailored "towards the truly risky microorganisms instead of trying to create all-encompassing regulatory oversight". The other commenter (#13) recommended that EPA "refocus the oversight of TSCA to the original intent of the legislation" and extend TSCA oversight only to those microorganisms used to produce "potentially toxic and otherwise unregulated chemicals."

EPA response:

EPA disagrees with the commenters who expressed concern that EPA's authority under TSCA to regulate microorganisms was "ambiguous" or legally vulnerable, and who suggested legislative amendments. As noted in the 1986 Coordinated Framework for Regulation of Biotechnology, an interagency workgroup concluded that existing health and safety laws provided adequate authority to meet current and prospective regulatory needs (51 FR 23303, June 26, 1986).

As indicated in the preamble to the proposed rule (59 FR 45527, September 1, 1994), EPA believes that the TSCA § 3(2) definition of "chemical substance" gives EPA authority to review living microorganisms under TSCA. Section 3(2) defines "chemical substance" as "any organic or inorganic substance of a particular molecular identity, including (i) any combination of such substances resulting in whole or in part from a chemical reaction or occurring in nature...." All living organisms, including microorganisms, fall within this definition. Organisms are composed of chemical substances, both organic and inorganic, which are of a "particular chemical identity". They can be grouped into classes of chemical substances, such as carbohydrates, fats, proteins, nucleic acids, water, etc.. As with all classes of substances composing organisms, genetic material meets the definition of chemical substance. Genetic material is composed of nucleic acids (ribonucleic acid (RNA) and deoxyribonucleic acid (DNA)). Moreover, the classes of chemical substances named above arise from a "chemical reaction" or occur naturally and all of the processes occurring in living organisms are, at base, chemical reactions. Thus, as organisms contain substances of particular chemical identities from all of the classes named above, they are clearly "combinations of such substances".

EPA agrees with the commenter (#13), who stated that "DNA, RNA and living things in general are composed of biochemicals produced through biochemical reactions which are driven by enzymes." However, biochemicals are a subclass of chemical substances, and biochemical reactions are chemical reactions. At the most fundamental level, drawing a distinction between chemicals and biochemicals is artificial. Enzymes, a type of biochemical, are catalysts of chemical reactions. The chemical reactions effected by enzymes can be effected by other means. While

there may be differences in efficiency and conditions of reaction between enzymes and other catalysts, the outcome in terms of action is the same.

The conclusion that genetic material, as well as living microorganisms, are "chemical substances" under TSCA is consistent with the legislative history, which makes it clear that the term "chemical substance" is to be interpreted broadly. The 1976 House Committee report notes that "the Committee recognizes that basically everything in our environment is composed of chemical substances and therefore the definition of chemical substance is necessarily somewhat broad." In recognition of this fact, the statute explicitly extends the term "chemical substance" to naturally occurring substances (§ 3(2)(A)).

Congress explicitly provided a number of exclusions from the definition of chemical substance, and nothing in the statute or the legislative history indicates that Congress intended to exclude microorganisms from the definition of chemical substances or from TSCA jurisdiction. Nor does the definition of chemical substance distinguish between potentially risky and safe substances. The legislative intent behind the breadth of the definition is tied up in the motivation underlying TSCA's enactment. TSCA was enacted to ensure that potential harmful effects of all chemicals were discovered and prevented before substantial segments of the population or environment were exposed. The House Report notes that the basis for TSCA was the concern that:

[a] vast volume of chemicals have, for the most part, been released into the environment with little or no knowledge of their long-term health or environmental effects. As a result, chemicals currently in commercial and household use are now being found to cause or contribute to health or environmental hazards unknown at the time commercial use began....As the preceding examples [of vinyl chloride, asbestos, and PCBs] indicate, it is often many years after exposure to a harmful chemical before the effects of its harm become visible. By that time, it may be too late to reverse those effects....Because diseases caused by environmental factors such as chemicals are often not susceptible to direct medical cure, there is an urgent need to prevent such chemically caused harm....Similarly the environmental harm caused by chemicals, like health effects, may be irreversible, and prevention of such harm is also urgently needed.

H.R. Rep. No. 1341, 98th Cong., 2d Sess. 3-4 (1976). The primary mechanism by which this was to be accomplished was to require EPA to review all new chemical substances before they were commercially manufactured, without regard to whether the chemicals were thought to be potentially risky, to determine whether they "may present an unreasonable risk of injury to health

and the environment". It would be inconsistent with this design to restrict the statute's jurisdiction in such a way to permit the unlimited manufacture of and human and environmental exposure to a class of substances.

Nor does EPA have a factual basis for such an exclusion; as a class, all microorganisms, like all chemicals, under certain circumstances, have the potential to present an unreasonable risk.

Microorganisms can induce adverse effects through a number of mechanisms. One mechanism is when the proliferating microorganisms grow in or on the target organisms' tissues and directly affect the target organism (pathogenicity). Microorganisms can also have deleterious effects when they produce toxins which can work when the organism producing the toxin grows outside of and at a distance from the target organism. For example, humans can be adversely affected by consuming foods containing the toxins produced by the bacterium *Clostridium botulinum*. The individual need not ingest the bacterium to be affected. And there are other, less direct ways in which a microorganism can affect other organisms; for example, by altering the habitat and thereby indirectly affecting populations occupying the same habitat. An example of this type of activity is when algal blooms in lakes produce anoxic conditions that can lead to disease symptoms and death in oxygen-requiring populations in the lake. In this case, the adverse effects are due to anoxia indirectly caused by the microbial population imbalances. Indirect effects can also be caused by microbial populations producing inorganic chemicals that can induce abnormal physiological processes in other organisms. For example, hydrogen sulfide produced by microbial populations in sediment can accumulate to concentrations toxic to other organisms. (Ref. 1A)

EPA's jurisdiction over microorganisms is also consistent with the Congressional intent that TSCA provide authority over substances not specifically subject to other health and environmental laws which address pesticides, foods, drugs, cosmetics, and medical devices. Certain uses of microorganisms now being developed do not fall within the above product categories. In general, the intended use of a microorganism, or any other chemical substance, determines whether it is subject to TSCA or to other laws. TSCA specifically excludes microorganisms that are manufactured, processed or distributed in commerce solely for use as a food, food additive, drug, cosmetic, or medical device, as those terms are defined under the FFDCA, and microorganisms that are manufactured, processed, or distributed in commerce solely for use as pesticides, as defined under FIFRA. With the exception of those exclusions, all microorganisms produced for environmental, industrial, or consumer uses fall within the scope of TSCA's jurisdiction. However, the fact that a microorganism falls under TSCA jurisdiction does not necessarily mean that its manufacturer will be required to submit a premanufacture notice, nor that the microorganism's manufacture or use will be restricted under TSCA. As will be discussed further below, in Unit II.D.3., TSCA authorizes EPA to receive notification regarding "new" chemical substances and it is in this process that EPA considers the risks of a chemical substance and whether restrictions on its manufacture, processing, distribution in commerce, use, or disposal are necessary.

Not only is EPA's interpretation that microorganisms fall within the definition of chemical substances consistent with the statute's legislative intent, EPA has consistently interpreted TSCA to cover microorganisms for almost 20 years. In the 1977 Inventory Reporting Rules, EPA responded to a comment that commercial microorganisms should not be considered "chemical substances" under TSCA, by stating:

"The term chemical substance is defined to mean 'any organic or inorganic substance of a particular molecular identity including any combination...occurring in nature.' This definition does

not exclude life forms which may be manufactured for commercial purposes and nothing in the legislative history would suggest otherwise." (42 FR 64584-85, December 23, 1977).

And in fact, 192 microorganisms were reported to and listed on the original TSCA Inventory. EPA further discusses the 192 microorganisms listed on the original Inventory below in Unit V.B.4. EPA again clarified in both the "Proposed Policy Regarding Certain Microbial Products", 49 FR 50880 (December 31, 1984) in the Office of Science and Technology Policy's (OSTP) "Proposal for a Coordinated Framework for Regulation of Biotechnology", 49 FR 50856 (December 31, 1984), and the 1986 final "Statement of Policy; Microbial Products Subject to the Federal Insecticide, Fungicide, and Rodenticide Act and the Toxic Substances Control Act", 51 FR 23313 (June 26, 1986), in OSTP's "Coordinated Framework for Regulation of Biotechnology", 51 FR 23302 (June 26, 1986), that living microorganisms would be considered chemical substances potentially subject to TSCA (49 FR 50886887, December 31, 1984; 51 FR 23315, June 26, 1986).

Further, EPA's exercise of jurisdiction over microorganisms has been consistent with its coverage under TSCA of traditional chemical substances similarly used. For example, TSCA has always covered traditional chemicals used as fertilizers. More recently EPA has reviewed under TSCA submissions for microorganisms used as biofertilizers, specifically for enhanced nitrogen fixation. Additionally, EPA has reviewed submissions for both traditional chemicals and microorganisms used as intermediates to produce specialty chemicals, such as enzymes and pesticides.

EPA disagrees with the commenters (#13, 37) who in essence suggest that only microorganisms that are known to pose risk, or that are used to produce "potentially toxic and otherwise unregulated chemicals" should be subject to TSCA. As discussed above, the statutory definition of chemical substance does not exclude potentially "safe" or "non-toxic" chemicals, although it does specifically exclude pesticides and products subject to the FFDCA. Nor does it limit coverage only to "risky" chemicals, or to those known to pose unreasonable risks. Further, EPA does not interpret the definition to incorporate an exclusion for all chemical substances that EPA has not specifically found are "potentially toxic and otherwise unregulated" or that potentially present an unreasonable risk. Congress explicitly rejected bills that restricted EPA's responsibility to reviewing only "potentially dangerous chemicals" (E.g., H.R. Rep. No. 1341 at 82 and 140; Cong. Rec. H8803-8863; Cong Rec. S4397-4432). Accordingly, EPA believes that the statutory definition should be interpreted broadly.

As a practical matter, EPA could not determine which microorganisms were potentially toxic, or would produce potentially toxic chemicals, without some information on the microorganism. As with traditional chemical substances, all microorganisms are "potentially toxic", depending on variables such as the concentration, the route of exposure, or the sensitivity of the exposed organisms. Similarly, all living organisms produce a range of substances which could be "toxic" under appropriate circumstances. EPA cannot determine whether a chemical substance may present a risk without some information on the context and manner in which it will be used, and EPA does not currently have the basis for determining *a priori* that a microorganism will not have

the potential to present a risk, when commercially manufactured, processed, distributed, or disposed.

Despite the commenter's (#13) implication, it is not merely the chemicals produced by microorganisms that may pose an unreasonable risk of injury to human health or the environment; the microorganisms themselves have the potential to present an unreasonable risk of injury to the environment from mechanisms other than toxin production. For example, pathogenic microorganisms can produce factors that are not “toxins” but which are important to the pathogenic process. In another example, a microorganism could displace or "out compete" existing organisms from an ecosystem, e.g., as happens with algal blooms. There is also the risk that a microorganism could transfer genetic material to other organisms, thereby conferring traits which would be harmful in the general population. These risks would not be addressed by the commenter's (#13) suggestion. EPA currently addresses many of the risks posed by chemicals produced by microorganisms in its traditional chemicals program. Chemical substances produced by microorganisms and used as products subject to TSCA are subject to review under TSCA § 5, separately from the microorganisms that produce them. Such chemical substances are subject to the same requirements and procedures as chemicals produced by other means.

Further, even if EPA were to adopt the commenters' suggested restrictions, few, if any, microorganisms would be excluded. As noted above, all microorganisms are potentially toxic and produce a range of potentially toxic substances. It is also unclear to what extent the criterion that the chemical substance be "otherwise unregulated" would exclude any microorganisms; TSCA was intended to apply when other statutes were insufficient to prevent or reduce an unreasonable risk of injury.

At most, EPA could exempt microorganisms from the § 5 review process, but as discussed below at Unit II.D.3., EPA can grant such an exemption only to the extent that EPA can determine the microorganisms will not present an unreasonable risk of injury to health or the environment.

B. APPLICABILITY OF TSCA TO PLANTS AND ANIMALS

In the proposal, EPA indicated that based on its interpretation of the term "chemical substance" to include living organisms, plants and animals could also be covered under TSCA. EPA stated that the rulemaking was limited to microorganisms. However, EPA also reserved authority under TSCA to screen transgenic plants and animals in the future as needed.

Comments:

EPA received nine comments (#8, 18, 25, 29, 32, 33, 35, 36, 40) on the applicability of TSCA to plants. Two commenters (#29, 35) supported EPA's intent to retain authority to screen transgenic plants. Seven commenters (#8, 18, 25, 32, 33, 36, 40) strongly opposed regulation of plants under TSCA and indicated their belief that plants were adequately covered by authorities under FDA and USDA.

EPA response:

The final rule is limited to coverage of living microorganisms under TSCA. Should EPA determine that TSCA coverage of organisms other than microorganisms is necessary such organisms that will be addressed in a separate document, at which time the public will be given further opportunity to comment on any proposed changes to the TSCA biotechnology program.

C. GENERAL TYPES OF PRODUCTS SUBJECT TO TSCA

EPA reiterated in the proposed rule that the definition of "chemical substance" in TSCA excludes pesticides, tobacco and tobacco products, food, food additives, drugs (including human drugs, animal drugs, and animal biologics), cosmetics, and substances that are used as medical devices (59 FR 45527, September 1, 1994). Microorganisms may be used as intermediates to produce chemical substances that are in turn used as products subject to TSCA or other statutes. The Food and Drug Administration (FDA) considers intermediates used to make products subject to FFDCA to be components of these products, and therefore they are excluded from regulation under TSCA. All other intermediates, including pesticide intermediates, are presumptively subject to TSCA jurisdiction.

Four commenters (31, 13, 28, 33) raised issues regarding the types of products covered under TSCA and the overlap with other authorities, specifically FFDCA and FIFRA. One commenter (31) requested EPA clarification regarding TSCA oversight of certain products. Three commenters (13, 28, 33) took issue with EPA regulation under TSCA of specific microorganisms which EPA has determined are pesticide intermediates under TSCA.

1. Clarification of TSCA oversight.

Comments:

One commenter (#31) requested that EPA confirm that this rule does not cover:

1.Non-FIFRA registered pesticides, R&D pesticides and pre-EUP pesticides which are synthesized or manufactured, evaluated (including laboratory and field trial evaluation):

a. for export., See: FIFRA Export Policy, 40 CFR 168.65, 58 Fed Reg 9061 (2118193); Attachment I (EPA PC #2289; 5/6/93 letter to EPA);

b.for domestic field trials in accordance with 40 CFR 172 (EPA-FIFRA notice requirement for small scale field testing to evaluate pesticidal activity/use as pesticides)

2.Research and development microorganisms; raw materials, components, intermediates and catalysts used in the synthesis and/or manufacture of research and commercial foods, drugs, cosmetics, medical devices, 42 Fed Reg 64572, 64586 (12/23/77); PC 1970 [Attachment 2]; 1986 EPA correspondence to DuPont [Attachment 2A];

3.Seeds--including
a.seeds which are transformed with DNA cloned in microorganisms, and
b.seeds which result from plant progeny, 59 Fed Reg at 45526 ("plants and animals are not subject to this rule");

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5 .Plants and seeds, including *but not limited to* plants and seeds regulated by USDA and by APHIS." (Italics in original).

EPA response:

TSCA § 3 explicitly ties TSCA jurisdiction to the determination of the use for which the microorganism is manufactured, processed, or distributed in commerce. A microorganism will not fall within TSCA's jurisdiction insofar as it is actually manufactured, processed or distributed in commerce for use as a pesticide, food, food additive, drug, cosmetic, or device. Microorganisms manufactured for multiple uses would be subject to TSCA for only those uses that fall within the scope of TSCA's jurisdiction.

To be excluded from TSCA jurisdiction as a pesticide, a microorganism must both be a pesticide, as defined by FIFRA, and must be "manufactured, processed, or distributed in commerce for use as a pesticide". Thus, a substance that can act as a pesticide will be subject to TSCA jurisdiction if it is not "manufactured, processed, or distributed for use as a pesticide".

Under FIFRA, whether a microorganism is a pesticide depends on a determination of intent; § 2(u) of FIFRA, in relevant part, defines a pesticide as:

(1) any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest, and (2) any substance or mixture of substances intended for use as a plant regulator, defoliant, or desiccant,...

The requisite intent may be demonstrated by direct evidence, or may be inferred from various circumstances. Examples of direct evidence of an intent to manufacture, sell or distribute a microorganism as a pesticide include the following: (1) submission of a notification under 40 CFR § 172.3 for a field test for a microbial pesticide; (2) an application for an experimental use permit under 40 CFR § 172.4; (3) an application for registration under 40 CFR part 152; or (4) submission by an exporter of a Purchaser Acknowledgment Statement under 40 CFR § 168.75.

This rule does not apply to microorganisms for which any of the four documents listed above have been filed, to the extent that the microorganisms are actually manufactured, processed, or distributed in commerce solely for use as a pesticide. Thus, in response to the commenter (#31), EPA will confirm that all pesticides manufactured solely for domestic field trials for which a microbial notification was submitted in accordance with 40 CFR § 172.45, or manufactured solely for export, for which an export notification has been filed pursuant to 40 C.F.R. § 168.75, will not be subject to this rule. However, the determination of whether a particular microorganism is a pesticide will, to some extent, necessarily be a case-by-case decision that turns on the facts surrounding the individual substance. Prior to actions demonstrating that a microorganism is a pesticide and actions demonstrating an intent to manufacture, sell or distribute it in commerce solely for use as a pesticide, a microorganism, including those in the process of research and development, will be presumed to be a chemical substance within the meaning of TSCA, and will be subject to TSCA and its implementing regulations.

Microorganisms used to produce foods, food additives, drugs, and cosmetics would not be subject to TSCA because they are within FDA's jurisdiction under FFDCA. The definitions in § 201 of the FFDCA provide that a substance that is intended for use as a component of a food, food additive, drugs, cosmetic, or device is encompassed within the meaning of such terms respectively, and is subject to regulation under the FFDCA.

EPA has previously discussed these issues at length in the 1977 Inventory Reporting Regulations (42 Fed. Reg. 64586 (December 23, 1977)), and in several other EPA documents.

As discussed above, EPA has limited this rulemaking to coverage of living microorganisms under TSCA. Therefore, plants and seeds would not be subject to this rulemaking. However, as EPA stated in its proposed rule, "microorganisms into which plant or animal gene segments are intentionally incorporated would be considered microorganisms potentially subject to TSCA". 59 FR 45526, 45527 (September 1, 1994).

2. Coverage of pesticide intermediates.

Comments:

Three commenters (#13, 28, 33) took issue with EPA's coverage under TSCA of specific microorganisms that EPA has determined are pesticide intermediates. The commenters claimed

these microorganisms were never isolated and thus should not be subject to TSCA requirements. One commenter (#13) stated that microorganisms used to produce pesticides should be regulated only under FIFRA:

Historically, the Agency has regulated chemical intermediates used to produce pesticides under TSCA, while pesticide active ingredients *per se* are specifically exempted from TSCA regulation and are fully regulated under FIFRA. We understand that the major reason for such an approach is that pesticide intermediates are typically further reacted to produce pesticides, but they can be used potentially to produce chemical products other than pesticides. The regulatory authority of FIFRA does not cover these intermediates unless they are a part of an integrated manufacturing process used to produce a pesticide active ingredient, in which no intermediate is isolated in the process. We contend that the use of microorganisms to produce pesticides, whether genetically engineered or not, has no place within TSCA, because such processes must be very specific to the use of a recipient and vector system whose sole purpose is to produce a pesticide end product. These systems are not at all analogous to the production of intermediates in typical chemical synthesis and reaction processes.

The other two commenters (#28, 33) suggested that the tiered exemptions from MCAN reporting set forth in subpart G of these final regulations be extended to cover nonisolated intermediates such as the microorganisms described above.

EPA response:

EPA disagrees with the commenters who state that these microorganisms are not pesticide intermediates and should not be regulated under TSCA. Congress established EPA's authority to cover pesticide intermediates under TSCA and EPA has consistently interpreted TSCA to cover pesticide intermediates since 1977. Raw materials and intermediates produced or used in the manufacture of a pesticide, which are not themselves pesticides or which are not manufactured, processed or distributed in commerce for use as pesticides, are substances that can be regulated under TSCA.

Moreover, the commenter (#13) has misunderstood the interplay between TSCA and FIFRA jurisdiction over chemical substances. As discussed above, unless a microorganism both meets the definition of a pesticide, and is manufactured, processed or distributed for use as a pesticide, it is subject to TSCA. Pesticide active ingredients are neither *per se* specifically excluded nor exempted from TSCA regulation, nor are they always fully regulated under FIFRA. An active ingredient that has no significant commercially viable use as distributed or sold, other than use for pesticidal purposes or for manufacturing a pesticide, is regulated by FIFRA as a pesticide. An active ingredient that does not meet all of the requirements listed in 40 CFR § 152.15(b) will generally not be considered a pesticide, and therefore may be subject to TSCA.

Regarding the specific pesticide intermediates referred to by commenters, the registered pesticide is the killed microorganism, and because no evidence has been presented to demonstrate an intent

to sell, distribute, or use the live microorganisms as a pesticide, EPA considers the live microorganisms to be pesticide intermediates subject to TSCA. During the manufacturing process, the live microorganisms, which have been modified to contain genes which encode for the production of a toxin selective for lepidopteran insects, are killed. Only the killed encapsulated microorganisms containing the toxin gene sequences are regulated under FIFRA, as the live microorganisms are not intended to be used as pesticides, and are not sold or distributed as pesticides, but rather are intended to produce pesticides. Accordingly, the live microorganisms are appropriately covered under TSCA as pesticide intermediates. EPA has consistently interpreted TSCA to cover, as pesticide intermediates, precursor chemical substances consumed in reactions that produce active ingredients for use in pesticides.

Risk considerations also dictate that EPA address the living microorganisms. The potential exists for unintentional releases of the microorganisms and the microorganisms may be capable of surviving for long periods of time in the environment. Prior to inactivation, the microorganisms present distinct hazard and exposure issues that should be addressed in the manufacturing process. The live microorganisms are appropriately regulated as pesticide intermediates under TSCA.

EPA does not agree with the commenters who suggest that the live microorganisms be considered non-isolated intermediates and thus exempt from TSCA § 5. In 40 CFR § 720.3(w), a non-isolated intermediate is defined as a chemical substance "not intentionally removed from the equipment in which it is manufactured." This refers to chemicals that are created and consumed within the same vessel or system. For example, when two chemicals are reacted to produce a third, there may be intermediate products created that are not isolated from the process and are consumed in intermediate reactions. EPA believes that it is unlikely that intergeneric microorganisms would meet the § 720.3(w) definition of non-isolated intermediate because the live microorganisms are created and isolated outside the fermentation vat and then added to the vat. Given the present technology, it is unlikely that microorganisms will meet the definition of non-isolated intermediates. EPA believes that it will be necessary to construct and maintain most microorganisms separately and then introduce them to a fermentation vat for production.

EPA also does not agree with the commenters who suggest extending the tiered exemption to non-isolated intermediates. The tiered exemption, which will be discussed below in Unit III.C, is structured around three specific criteria. One of these criteria is that the recipient microorganism must be listed as an eligible candidate. At this point in time, no such intermediates are listed at §725.420. However, in conjunction with the tiered exemption, EPA has established a process at §725.67 whereby submitters can petition EPA to add a specific microorganism to the list of eligible recipients, if the microorganism meets the criteria for the exemption.

In summary, EPA believes that live microorganisms used as intermediates are appropriately regulated under TSCA, whether they are ultimately killed for use as pesticides or they are used solely to produce specialty chemicals which are subject to TSCA or FIFRA.

D. SCOPE

EPA proposed to retain for this rulemaking the 1986 policy statement interpretation of "new" microorganisms as intergeneric microorganisms not listed on the TSCA Inventory. EPA also proposed to retain the exclusion from the definition of "new microorganism," intergeneric microorganisms resulting solely from the addition of well-characterized, non-coding regulatory regions. In the proposal EPA also discussed mobile genetic elements (MGEs) and how it would apply its MGE policy to the interpretation of "new" microorganisms for the purposes of TSCA § 5. The proposal defined "MGE" as an element of genetic material that has the ability to move genetic material within and between organisms. As part of EPA's MGE policy, the MGE is identified as belonging to the genus from which it was originally isolated. EPA indicated that it might decide to reconsider its interpretation of "new" microorganism in a separate rulemaking and requested comment on whether it should explore alternative interpretations.

EPA received 17 comments on scope of oversight. Two commenters (#12, 36) supported EPA's interpretation of "new" microorganisms as stated in the proposal without additional modifications. The 15 other commenters requested clarification on the use of taxonomy as a regulatory standard; suggested specific modifications to the intergeneric scope; and/or requested a more "risk-based" approach to oversight.

1. Retention of "intergeneric" interpretation

Comments:

Of the 17 comments received on scope of oversight, only four commenters strongly opposed the intergeneric scope and supported another approach, while 13 commenters expressed some level of support for intergeneric, albeit with some modifications. One commenter (#12), while acknowledging that there are problems with an intergeneric scope, indicated: "[n]evertheless, no one has proposed a clearly superior scope, despite years of discussion and debate. Adoption of the intergeneric scope by EPA at this time is logical."

EPA response:

EPA is retaining its interpretation of "new" microorganisms as stated in the 1986 policy and the proposed rule. Under that interpretation, microorganisms resulting from deliberate combinations of genetic material from organisms classified in different genera constitute "new" microorganisms subject to § 5 reporting requirements. EPA terms such microorganisms intergeneric. EPA has edited the intergeneric definition at § 725.3 to clarify which microorganisms are included and excluded in the definition. The definition reads as follows:

"*Intergeneric microorganism* means a microorganism that is formed by the deliberate combination of genetic material originally isolated from organisms of different taxonomic genera.

(1) The term "intergeneric microorganism" includes a microorganism which contains a mobile genetic element which was first identified in a microorganism in a genus different from the

recipient microorganism.

(2) The term "intergeneric microorganism" does not include a microorganism which contains introduced genetic material consisting of only well-characterized, non-coding regulatory regions from another genus."

Intergeneric scope. EPA has decided to retain its 1986 interpretation of “new microorganisms” as those microorganisms resulting from the deliberate combining of genetic material from organisms classified in different genera because of the degree of human intervention involved, the significant likelihood of creating new combinations of traits, and the greater uncertainty regarding the effects of such microorganisms on human health and the environment. This approach, based on a taxonomic standard, both identifies a group of microorganisms whose behavior in the environment poses significant uncertainty, which therefore warrant regulatory review under TSCA § 5, and provides a way of defining "new" microorganisms under TSCA § 5.

Section 5 requires all manufacturers of new chemical substances to submit information to EPA 90 days before commencing commercial manufacture, to permit EPA to examine whether they may present an unreasonable risk of injury to health and the environment. As discussed in Unit II of the Response to Comments Document, the rationale for the requirement was to have EPA attempt to resolve the uncertainties surrounding the class of new chemical substances--specifically, whether they were likely to cause unreasonable risks before they were introduced into the environment.

As noted previously, EPA has attempted, where possible, to make its regulatory program for microorganisms consistent with its TSCA program for traditional chemical substances. As with traditional chemical substances, any microorganism that is not on the Inventory is “new” and is therefore subject to premanufacture reporting. In compiling and maintaining the TSCA Inventory, EPA distinguishes between “new” chemical substances, which are subject to PMN, and “naturally occurring” substances, which are not. Under the Inventory reporting rules, naturally occurring substances and substances derived from nature with limited human intervention are considered to be automatically included on the Inventory, and thus are not “new”. 40 C.F.R. §710.4(b).

This approach reflects a general philosophy that human intervention at a relatively simple level does not remove a substance from the category of naturally occurring. The act of mechanically isolating a substance from nature does not alter its status as “naturally occurring” or make it subject to § 5 reporting. In short, under EPA’s traditional chemical program, two principles must be considered in determining whether a substance is exempt from § 5 reporting by virtue of being naturally occurring. First it must be derived from nature. Second, the extent of human intervention in producing it must be limited.

The Agency believes that similar logic should be used to determine whether a microorganism is “new”. In principle, naturally occurring microorganisms are those that (1) exist as a result of

natural events or processes, or (2) have been developed as a result of limited manipulation of natural processes. For example, normal events of reproduction or evolution do not produce "new chemical substances" subject to § 5 reporting any more than chemical reactions in nature, unmediated by humans, create "new chemical substances". Similarly, human exploitation of natural reproductive processes, as in the case of traditional animal and plant breeding, does not create a "new chemical substance", any more than does extracting a nonliving substance by manual, mechanical or gravitational means from a naturally occurring substance. Therefore, "naturally occurring microorganisms" are automatically listed on the TSCA Inventory, and are not subject to this rule.

EPA has defined "new microorganisms" as those microorganisms formed by combining genetic material from organisms classified in different genera, i.e., intergeneric. EPA believes these microorganisms would have a higher potential for exhibiting a new trait or combinations of traits, and thus are less likely to occur through natural processes in nature.

A trait is the ability to perform a function; e.g., to combine two atoms of hydrogen with an atom of oxygen to make water. In a living organism, the means of effectuating a function is usually a protein (enzyme). The information necessary for an organism to make and control the activity of a protein is encoded in the organism's genetic material. The genetic information an organisms possesses therefore determines what substances it will make and traits expressed and thus how it will look and behave.

EPA decided that a standard based on taxonomy was an appropriate method of defining a "new microorganism". Taxonomy is a system of orderly classification of organisms according to their presumed natural relatedness. Organisms that are more closely related are more likely to have the same traits than are microorganisms that are more distantly related. EPA also decided that genus was the appropriate taxon within the taxonomic system established by Whittaker (cited in Atlas and Bartha (1987)(Ref. 24)) to describe a "new microorganism" because a combining event involving the genetic material of organisms classified in different genera presents a sufficiently high potential of the resulting microorganism exhibiting a new trait or new combinations of traits. Since the organisms contributing genetic material to intergeneric microorganisms are, in general, more distantly related than the microorganisms contributing genetic material to intrageneric microorganisms, intergeneric microorganisms have a higher potential of exhibiting a new trait or new combinations of traits.

In addition, EPA has determined that intergeneric microorganisms, since they are more likely to exhibit "new traits", are not as likely to be found occurring naturally and thus can be seen as the product of a substantial degree of human intervention.

A scope based on a taxonomic standard such as intergeneric has certain advantages. A taxonomy based scope relates directly to the potential of the resulting new microorganism to display a new trait or new combinations of traits, since organisms that share a close evolutionary ancestry are more likely to have traits in common than those that are more distantly related. In addition, the

taxonomy standard is independent of the technology used to create the microorganism. A number of techniques may be used to produce intergeneric microorganisms. Any intergeneric microorganisms created by techniques developed in the future would also be subject to these regulations. Finally, since intergeneric microorganisms are more likely to express new traits, the behavior of these microorganisms is significantly less predictable than the behavior of intrageneric microorganisms. EPA believes this measure of unpredictability warrants a level of regulatory scrutiny.

Taxonomy reflects current scientific observations about phenotypic, and to a certain extent, genotypic, differences between organisms. Although subject to periodic revision within the scientific community, taxonomy is a common language used by scientists. Basing the standard for interpreting "new" for microorganisms on an existing system for categorizing organisms obviates the need to create another system for determining if a microorganism is subject to reporting under TSCA § 5. Taxonomy is understood by the regulated community and its use imposes little, if any, additional burden to determine whether a microorganism is new.

For circumscribing what is "new" for TSCA § 5 purposes, microbial taxonomy is a relatively clear and objective criterion for determining the scope of oversight, and thus provides clarity for the regulated community and provides an enforceable criterion. Taxonomic designations provide a widely available standard and point of reference. It is reasonable to expect a manufacturer to use the taxonomic literature and/or taxonomists to determine currently accepted names of organisms they wish to utilize. Once a manufacturer knows the genus of these microorganisms, he or she can readily determine whether a microorganism is intergeneric and thus whether it is "new" within the § 5 context.

EPA recognizes that taxonomy, particularly microbial taxonomy, is subject to change and that new information concerning organisms' properties and relationships could alter taxonomic designations. In recent years, new tools have become available to microbial taxonomists which have allowed them to clarify phylogenic relationships among microorganisms. Some microbial genera are highly defined and consist of closely related members which are likely to share common information in their genetic material. However, other microbial genera may consist of members more closely related to microorganisms classified in other genera than to each other. While reorganizations could result in changes in taxonomic designations for some microorganisms in the short term, it should result in greater stability in the various taxa in the long term. EPA anticipates that as reclassifications occur in the scientific community, the intergeneric standard will become a better reflection of the probability of new traits or new combination of traits resulting from the deliberate combining of genetic material. However, even under current taxonomic designations, gene exchange is generally less likely to occur naturally among members of different microbial genera than among members of the same genus, and this suggests a new trait or new combinations of traits are more likely to occur when genetic material from microorganisms in different taxonomic genera are combined. Moreover, the probability of a new trait or new combination of traits occurring increases when the organisms combining genetic material are more distantly related; e.g., even among the microorganisms, bacteria classified in different genera are

more likely to share common traits than bacteria and fungi, and bacteria classified in different genera even more likely to share traits than bacteria with plants and animals. While taxonomic reorganizations could affect the status, for TSCA purposes, of some microorganisms formed by combining genetic material from some relatively closely related microorganisms, the TSCA § 5 status of microorganisms formed by combining genetic material of more distantly related organisms is unlikely to be affected. These considerations suggest that while taxonomy may not be a perfect standard, its use is likely to capture for review those microorganisms with a higher probability of displaying new traits or new combinations of traits. EPA discusses in other units of this Response to Comments document how it will accommodate within its regulatory structure reclassifications of microorganisms into new or different taxa.

EPA believes that on whole, the intergeneric definition generally captures for review microorganisms with a higher potential for displaying a new trait or new combination of traits. While this approach does have some drawbacks, EPA believes that its procedures are sufficiently flexible to accommodate these drawbacks, and that the advantages to using the intergeneric definition outweigh the disadvantages.

EPA inserts the term "originally isolated" into the definition of intergeneric to clarify that genetic material belongs to the genus from which it was originally isolated. For example, if a sequence of genetic material from microorganism A is introduced into microorganism B, subsequently transferred from microorganism B to microorganism C, the manufacturer or developer must consider the genera of microorganisms A and C in determining the status of the microorganism resulting from the second combining event described above.

Mobile genetic elements. In the proposal (59 FR 45528), EPA also discussed mobile genetic elements (MGEs) and how it would apply its MGE policy to the interpretation of "new" microorganisms for the purposes of TSCA § 5. EPA has retained the policy and incorporated it in its definition of intergeneric microorganism. MGEs, which are elements of genetic material such as plasmids and transposons, may in nature move within or among organisms and may carry with them and transfer genetic material in addition to their own. MGEs are vectors that move genetic material among organisms. These elements may move across taxonomic boundaries and therefore are not a constant part of the genome of one particular taxonomic group or another.

After publication of the 1986 policy statement describing EPA's intergeneric interpretation, several producers of microorganisms inquired about the status under TSCA of microorganisms containing MGE material. Therefore, it was necessary for EPA to develop an approach for addressing MGEs under the intergeneric interpretation. In keeping with its intergeneric definition which focused on the origin of the introduced genetic material, EPA decided that microorganisms would be considered intergeneric if they contained an MGE first identified in a microorganism in a genus different from the recipient microorganism genus. Microorganisms would be considered intrageneric, and not new, if the MGE was first identified in a microorganism in the same genus as the recipient. EPA has continued to use this policy regarding MGEs to assist in determining whether a microorganism is intergeneric.

The issue of whether the MGE may be indigenous to the recipient genus is not considered in EPA's approach to determining whether the final microorganism is inter- or intrageneric. The major consideration is the source of the organism in which the MGE was first identified. The source of the organism in which the MGE was first identified may be determined by a search of relevant published scientific literature or by reviewing available databases such as GENBANK. Such a literature or database reference is often the first to name, and possible describe, the MGE. Subsequent references postdating this first reference are frequently not relevant for determining the intergeneric status of the MGE, since after isolation an MGE is often transferred to a different taxon where it can be more easily maintained and studied. Although EPA recognizes that MGEs may occur in more than one genus in nature EPA believes that for the moment use of the source of the organism in which the MGE was first identified for classifying MGEs provides the most straightforward regulatory approach under its intergeneric definition. EPA will continue to use this approach until it can reevaluate the status of MGEs within an intergeneric standard in a future rulemaking. EPA has included a statement about MGEs in its definition of intergeneric microorganisms in this final rule.

Well-characterized, non-coding regulatory regions. In the 1986 policy statement and in the proposed rule, EPA excluded from the definition of intergeneric microorganisms, those microorganisms that resulted from the addition of intergeneric material that is well-characterized and contains only non-coding regulatory regions such as operators, promoters, origins of replication, terminators, and ribosome-binding regions. Where only regulatory material is transferred, no distinctly new combinations of traits are introduced. Instead, existing traits in the receiving microorganisms are amplified or changed quantitatively. Therefore, EPA believes that microorganisms formed only through intergeneric transfer of well-characterized, non-coding regulatory regions should not be considered "new" microorganisms under TSCA § 5. EPA emphasizes that this exclusion applies only to intergeneric microorganisms that have resulted solely from the addition of well-characterized, non-coding regulatory regions. If the final microorganism contains any regions from organisms of other genera that do not meet this restriction, such as coding regulatory regions or any poorly characterized regions, the microorganism is considered new and is subject to these regulations.

2. Clarification regarding taxonomy

Comments:

Three commenters (#18, 30, 37) were concerned about using taxonomy to define scope because of the continual changes in microbial systematics. They requested that EPA indicate which of several taxonomic systems EPA would accept in determining whether a microorganism was intergeneric and how changes in taxonomic classification will be accommodated. Two commenters (#18, 30) requested that EPA indicate how it will classify totally synthetic sequences.

EPA response:

The intergeneric definition is based on taxonomic designations. While imperfect in many ways, taxonomy appears to provide the best available standard for "dissimilarity" among organisms. Although subject to periodic revision within the scientific community, taxonomy reflects the most recent scientific observations about phenotypic and genotypic differences between organisms. EPA believes that the taxonomic status of most organisms relative to the recipient organism will be clear. However, EPA recognizes that changes in taxonomic designations for microorganisms may occasionally create less clear situations. Nonetheless, EPA expects that organisms being used in biotechnology will have or can be assigned clear taxonomic designations and that it should not be difficult to determine whether a microorganism is intergeneric. However, submitters who are uncertain as to whether their microorganism would be considered intergeneric should consult EPA.

Several commenters requested that EPA acknowledge the need for flexibility in taxonomic designation to accommodate the changes made, and being made, in classification of microorganisms. EPA agrees with the sentiment in these comments. The technology of microbial identification is improving rapidly, and as a result, major modifications in taxonomic organization of microorganisms have taken place recently, such as the recent subdivisions of the genera *Pseudomonas* and *Bacillus*, each into several new taxonomic units that include new additional genera. Additional changes can be expected in the future as well. These modifications of taxonomies have made possible the classification of previously difficult to categorize strains and have employed techniques that utilize genetic as well as phenotypic methods. EPA acknowledges that this development of systematics has led to multiple synonyms for some species, some used concurrently, such as the former *Pseudomonas* and the current *Burkholderia cepacia*. It is reasonable to expect that submitters will be aware of the most current designations for their strains, but it is possible that new developments will leave some species in transition; i.e. new

designations may be proposed, but not yet fully accepted by the microbial systematics community, such as for example the recently named *Sinorhizobium meliloti*. EPA will take into account changes in systematics as they occur.

As taxonomies change, EPA agrees that submitters may use alternative designations for their species when appropriate. EPA must know, however, which classification scheme was employed by the submitter in identifying the organisms used as DNA donor and recipient. That will allow EPA to relate the submitter's name for its microorganism with current terminology, if the names differ. When both are known, EPA will include the new and formerly accepted designations in its species descriptions. In some cases, the revised taxonomies do more than merely change a species or genus name. Often taxa are subdivided because the original grouping combined disparate organisms on the basis of a few remarkable features, such as with the genera *Pseudomonas* and *Bacillus*. Conversely, sometimes taxa have been improperly separated based on a few features, e.g. *Pseudomonas gardneri* and *Xanthomonas campestris.* Modern methods are revealing these problems and resulting in many taxonomic reorganizations. EPA will need to determine the appropriate new taxonomic affiliation of a submitter's organism under such

circumstances and may require some supplemental information to permit that determination. It is not intended that these additional data requirements be burdensome, but it is necessary to achieve a designation that unambiguously and accurately describes the submitted organism.

EPA acknowledges that there is a wide range of methods used to identify microorganisms and that these can provide conflicting identifications. EPA does not believe that it should prescribe any particular method for use in identifying submitted microorganisms. Rather, EPA believes submitters should use techniques that are appropriate for the species. Some species are readily identified by simple techniques, whereas others may require a more complicated set of methods. Submitters may use methods of their choice, but should be able to document why individual identification methodologies were selected, so that EPA may review the information properly.

A few submitted microorganisms will not fit neatly into any current classification scheme. Correlation of these "transitional" strains with existing taxa should be illustrated where possible, but submitters should not assign the name of the closest classified taxon to a new strain, if identity with that taxon cannot be confirmed. Rather, it is appropriate to indicate that the submitted microorganism is not currently identifiable with any existing species (or genus) and that it may belong to a new, as yet undescribed, species. Provided accompanying documentation permits EPA to recognize that the submitted microorganism is unique and can be distinguished from other similar microorganisms, an uncertain species designation would be acceptable. An example of the type of information that would be useful to EPA in such circumstances is provided in Murray and Stackebrandt, 1995 (Ref. 1) which recommends use of the category *Candidatus* as the taxonomic status for uncultured procaryotic cells which lack information on characteristics for a complete description but for which information exists on more than a sequence. The article suggests that in addition to sequence information, phenotypic information such as structural, metabolic and reproductive features and the natural environment in which the organism can be identified be included. These kinds of data could also be provided to EPA as supplemental information under § 725.12(b) for new microorganisms which have uncertain taxonomic status for any of several reasons, including but not limited to being nonculturable.

If the taxonomic positions of some organisms are ambiguous or if the boundaries of a genus are in dispute, EPA expects the submitter to be aware of these controversies. If the taxonomy at the genus level is controversial such that microorganisms may be considered by some to belong to the same genus and by others to belong to different genera, the submitter must comply with the requirements of TSCA for intergeneric microorganisms or contact EPA for a case-specific determination. In general, submitters should expect that microorganisms will be considered intergeneric if the taxonomy of either source organism, at the genus level, is controversial.

In the case of chemically synthesized genes, EPA will follow a similar principle. EPA recognizes that the genetic sequence of the synthesized gene may be identical to a sequence known to occur in the same genus as the recipient microorganism, in which case the resulting products would be considered intrageneric. The manufacturer should be prepared to document how this determination was made. If the sequence of the synthesized gene is different or not known to be

identical to a sequence in the genus of the recipient microorganism, the resulting new microorganism would be considered intergeneric. Submitters in this category should consult EPA.

3. Modifications to scope

Comments:

Eleven commenters (#8, 15, 18, 23, 24, 25, 26, 28, 33, 34, 35) suggested that the intergeneric scope could be improved by additional modifications, primarily to the MGE policy and the exclusion for well-characterized non-coding regulatory regions. Three of these commenters (#28, 33, 34) felt that intergeneric should be related specifically to a phenotypic change. "The central focus as to newness should be on whether there has been an intergeneric transfer of a new phenotypic trait." (#33)

Two commenters (#8, 18) opposed the exclusion for well-characterized, regulatory regions on the grounds that the locus of insertion can have “unforeseen effects”. One commenter (#35) suggested that the terms "well-characterized" and "non-coding regulatory region" be separately defined in § 725.3, and suggested the following modified definition of “non-coding regulatory region”:

‘Non-coding regulatory region’ means a segment of genetic material which solely controls the activity of other regions that code for protein or peptide molecules or act as recognition sites for the initiation or termination of nucleic acid or protein synthesis.

Six commenters (#8, 15, 18, 24, 30, 35) felt that the intergeneric scope should be modified to take into account natural gene exchange among microorganisms in different taxonomic genera. Four commenters (#8, 18, 24, 35) suggested that EPA use the NIH Guidelines list of natural exchangers as a starting point to exclude natural exchangers. Seven commenters (#8, 18, 23, 26, 28, 33, 35) felt that the MGE policy was too restrictive. Three commenters (#8, 18, 35) suggested that exclusion of the natural exchangers from the intergeneric definition would improve the scope. Four commenters (#25, 28, 33, 35) suggested that EPA place on the Inventory the NIH list of certified host-vector systems. One commenter (#15) urged EPA to adopt a process-based scope focusing on microorganisms "produced by certain techniques that can be used to combine organisms [sic] that do not exchange genetic material in nature."

EPA response:

As discussed above, EPA will retain for the time being its intergeneric interpretation of "new" microorganisms. EPA will continue to exclude from the definition of "new" microorganism those microorganisms resulting from the addition of intergeneric material that is well-characterized and contains only non-coding regulatory regions. Additionally, EPA will retain its MGE policy, under which microorganisms will be considered "new" if the MGE was originally isolated from a

microorganism in a genus different from the recipient genus.

In response to comments, EPA has revised some of its definitions at § 725.3 to provide greater clarity for the regulated community. The word "introduced" has been added to subparagraph (2) in the definition of "intergeneric microorganism" to clarify that microorganisms which contain introduced genetic material consisting only of well-characterized, non-coding regulatory regions from another genus are not considered intergeneric for the purposes of TSCA § 5. EPA agrees with the commenter (#35) who suggested that "well-characterized" and "non-coding regulatory region" be separately defined. Therefore, the definition of "well characterized, non-coding regulatory region" has been deleted and the following definitions of "non-coding regulatory region" and "well-characterized" have been added to § 725.3:

"*Non-coding regulatory region* means a segment of introduced genetic material for which:

(1) The regulatory region and any inserted flanking nucleotides do not code for protein, peptide, or functional ribonucleic acid molecules.

(2) The regulatory region solely controls the activity of other regions that code for protein or peptide molecules or act as recognition sites for the initiation of nucleic acid or protein synthesis."

"*Well characterized* for introduced genetic material means that the following have been determined:

(1) The function of all of the products expressed from the structural gene(s).

(2) The function of sequences that participate in the regulation of expression of the structural gene(s).

(3) The presence or absence of associated nucleotide sequences and their associated functions, where associated nucleotide sequences are those sequences needed to move genetic material including linkers, homopolymers, adaptors, transposons, insertion sequences, and restriction enzyme sites.

While EPA agreed with the suggestion to use the language in §725.421(b) to define “well characterized”, EPA did not use the definition of “non-coding regulatory region” suggested by commenter #35 because the definition did not include the specific requirement that both regulatory regions and inserted flanking sequences be non-coding.

EPA developed the definition of “non-coding regulatory region” based on language pertinent to the non-coding aspect of the definition of “well-characterized, non-coding regulatory region” as originally contained in § 725.3 of the proposed rule. EPA believes that it is necessary to

specifically require that the regulatory regions be non-coding. As stated in the 1986 policy statement and in the proposed rule, EPA excluded from the definition of intergeneric microorganisms, those microorganisms that solely contained intergeneric regulatory regions that are well characterized and non-coding. Such intergeneric material would not introduce distinctly new traits or new combinations of traits. Rather, the level of expression of existing traits in the recipient microorganisms may be altered. By also including a restriction that the flanking sequences be non-coding, EPA is ensuring that persons will consider the nature of the flanking sequences associated with regulatory regions when determining their eligibility for the well characterized, non-coding regulatory region exclusion.

EPA has determined that for this final rule it will not reconsider, as requested by commenters #8 and 18, whether EPA should continue to exclude from the definition of intergeneric, microorganisms that result from the addition of material that is well-characterized and contains only non-coding regulatory regions. Under § 5 of TSCA, EPA determines whether a chemical substance is “new”, independently of the determination that the chemical substance or microorganism may present risks. As discussed above, in defining “new microorganism”, EPA focuses on the potential for expression of new traits or new combinations of traits. Where only well-characterized, non-coding regulatory material is transferred, no distinctly new traits are introduced. Instead, quantitative changes in existing traits in the recipient microorganism may occur. EPA recognizes that the insertion of well-characterized, non-coding regulatory regions may result in expression of previously cryptic regions. However, the genetic material in cryptic regions is present in the recipient and could be expressed in other members of the recipients species at any time naturally. A microorganism expressing such material as a consequence of the insertion of well-characterized, non-coding regulatory regions would thus not be “new” under

TSCA. In the proposed rule, EPA indicated that it may choose to reconsider its interpretation of “new microorganism” at a later time and in a separate rulemaking. The issue raised by commenters #8 and 18 will be reconsidered at that time.

Of the 17 comments received on scope of oversight, only four commenters strongly opposed the intergeneric scope and supported another approach, while 13 commenters expressed some level of support for intergeneric, albeit with some modifications. EPA believes that while there are problems with the intergeneric scope, as one commenter noted, "no one has proposed a clearly superior scope, despite years of discussion and debate" (#12).

Except for the minor modification to the definitions of "intergeneric microorganism," "well-characterized," and "non-coding regulatory regions" discussed above, EPA will make no changes at this time in its approach to defining new microorganisms subject to this final rule. EPA has determined that a separate rulemaking is needed for the development and implementation of significant modifications to the intergeneric scope. Possible modifications to the intergeneric scope need to be considered as a whole in order to develop an administratively feasible approach based on the most recent scientific knowledge. In addition, EPA believes that an additional opportunity should be provided to the public for notice and comment.

4. Alternative approaches to scope

Comments:

EPA received nine comments (#8, 13, 18, 24, 26, 30, 35, 37, 39) supporting a "risk-based" approach to scope of oversight. Five of these commenters (#8, 18, 24, 26, 35) indicated that although they understood the reason EPA has an intergeneric scope, they believe that EPA should work on developing a more "risk-based" interpretation focussing on the new phenotypic traits introduced into a microorganism. Four commenters (#13, 30, 37, 39) opposed the focus on "new" microorganisms and supported, instead, an approach based on “the characteristics of the product, not on the process used to develop the product" (#37).

EPA response:

As one commenter (#23) noted, "EPA has a need that is unique among the federal agencies regulating biotechnology. While other federal agencies must define 'risky' organisms as their threshold question, EPA has a different requirement under TSCA: the need to define new organisms." For the purposes of administering TSCA, EPA must decide what constitutes a "new" microorganism. TSCA § 3(9) defines a "new chemical substance" as a substance not on the TSCA Inventory of Chemical Substances compiled under TSCA § 8. Naturally occurring substances and substances derived from nature with limited human intervention are not explicitly listed on the Inventory but are considered implicitly to be on it, and thus are not "new." EPA believes that when genetic material has been combined among source organisms from different

genera (intergeneric), the resulting microorganism should be considered "new" because of the degree of human intervention involved, the significant likelihood of creating new combinations of traits, and the greater uncertainty regarding the potential risks of such microorganisms.

One commenter (#26) noted that "consideration of significant 'newness' of an organism is a separate issue from the question of the risk posed by the organism and that 'newness' is not necessarily the appropriate criterion for determining the relative risk of the resulting organism." EPA considers newness and risk in its TSCA § 5 program separately. TSCA § 5 notification is triggered by "newness"; it is not triggered by a determination that a risk is present. However, EPA believes a scope based on intergeneric addresses some elements of risk; because there is a significant likelihood that intergeneric microorganisms will exhibit new traits or combinations of traits, there is greater uncertainty regarding the behavior of such organisms and their potential risk.

Once a notice has been received, risk is an important component of the review process. EPA does not regulate new chemical substances unless it determines that they may present an unreasonable risk to health or the environment or where there will be substantial production of the substance and significant or substantial exposure to the substance. Once EPA has gained experience with or sufficient information about certain categories of microorganisms, it may use

TSCA § 5(h)(4) to develop exemptions from the reporting requirements of § 5. In developing its TSCA § 5 program for microorganisms, EPA has taken advantage of the flexibility afforded by TSCA § 5(h)(4) to establish exemptions which can be expanded as EPA gains additional experience reviewing intergeneric microorganisms. These exemptions are discussed in more detail below in Units III.C. and IV of this Response to Comments document.

III. REPORTING GENERAL COMMERCIAL USE OF MICROORGANISMS

A.MCAN PROCESS

The proposed rule is based on procedures that had been originally developed and promulgated for the TSCA § 5 program for traditional chemicals which EPA has operated for over a decade. For the convenience of users, EPA proposed a new part in the Code of Federal Regulations (CFR) which incorporates these procedures, and applies specifically to microorganisms (Part 725). Procedures from parts 720 (premanufacture notification) and 721 (significant new use notification) were placed in the new part 725 with some minor modifications to accommodate the specific characteristics of microorganisms. EPA indicated that it was not seeking comment on the procedures in proposed part 725 that were incorporated from parts 720 and 721 without significant modification.

In lieu of the PMN or SNUN described in part 720 and 721, respectively, EPA proposed in part 725 a requirement for submission of a Microbial Commercial Activity Notice (MCAN) by persons who intend to manufacture or import new living microorganisms, and by persons who intend to manufacture, import, or process microorganisms for a significant new use. Because EPA proposed a separate, less burdensome reporting process for R&D involving microorganisms (discussed in Unit IV. of this document), EPA expects that the MCAN would be submitted only for microorganisms for general commercial use. The purpose of EPA's review of MCANs is similar to EPA's purpose in reviewing Premanufacture Notices (PMNs) and Significant New Use Notices (SNUNs) submitted for traditional chemicals. The purpose of a MCAN would be to provide EPA with information necessary to identify and list a microorganism on the TSCA Inventory (if the microorganism is new) and to determine whether the microorganism may present an unreasonable risk of injury to human health or the environment.

EPA received nine comments (#8, 18, 25, 26, 28, 33, 35, 37, 39) on issues related to its notification processes for microorganisms at general commercial use. Three commenters (#26, 33, 39) had general comments about the process. Seven commenters (#8, 18, 25, 28, 33, 35, 37) had specific concerns about contract manufacturing, certain information requirements for the MCAN process, and the inclusion of requirements for byproducts.

1.General comments

Comments:

One commenter (#26) expressed support for the MCAN process, calling it "an improvement over existing practices." Another commenter (#33) suggested that it was time for EPA to "begin development of a standardized form for microbial products subject to notification." A third commenter (#39) indicated that its company followed the NIH Guidelines when using recombinant microorganisms to produce microbial products for general commercial use and was concerned that "numerous MCANs and exemptions may be necessary for biotechnology firms who work with a multitude of different intergeneric organisms."

EPA response:

EPA is not ready to develop a MCAN form at this time. With the promulgation of this rule, EPA will be codifying information requirements for biotechnology submissions for the first time. Thus, EPA's experience with the MCAN is somewhat limited. In addition, as discussed in other sections of this document, EPA plans to consider, at a later time, further modifications to its interpretation that intergeneric microorganisms are new chemical substances under TSCA § 5. After EPA has gained additional experience reviewing submissions under the requirements of Part 725 and has completed its modifications to other parts of its approach, EPA will consider developing a MCAN form.

A commenter (#39) expressed concern that many MCANS would need to be submitted by companies working with many intergeneric microorganisms. EPA does not believe that this would be the case. Companies who are developing a variety of related intergeneric microorganisms may file "consolidated" MCANs for several microorganisms which are similar in construction and use. A "consolidated MCAN" is defined in § 725.3. Persons who wish to manufacture or import two or more related new microorganisms should contact a member of the biotechnology staff in the New Chemicals Program at EPA to discuss the parameters of submitting a single consolidated MCAN for the related microorganisms. A consolidated MCAN cannot be submitted for an open-ended category of microorganisms. The submitter must identify each new microorganism individually, because each microorganism in the consolidated MCAN will be assigned a separate MCAN number.

A consolidated MCAN is suitable for microorganisms with the same or similar uses and for which there are similar test data or other information. For example, EPA has received consolidated submissions for intergeneric microorganisms when the submitters have developed a series of microorganisms where the recipient microorganism and the product use are the same but the introduced genetic material has varied.

EPA will not accept a consolidated MCAN unless the submitter has contacted EPA and obtained a prenotice agreement that the group of microorganisms is sufficiently similar that they are suitable for review in a consolidated MCAN. However, EPA encourages submitters to submit consolidated notices when appropriate; such notices will reduce the burden associated with preparing multiple § 5 notices based on similar or identical information. Additionally, the fee for a consolidated MCAN is the same as that for one MCAN, $2500 (see 40 CFR § 700.45(b)(2)(vi),

revised as part of this rulemaking). Consolidated MCANs also will facilitate the efficiency and consistency of EPA's review by allowing the staff to review related microorganisms, and the data and other information that are common to them, at the same time.

Separate from use of a consolidated MCAN, submitters are encouraged to identify related MCANs in their submissions and to provide the basis for the claimed relationship. For example, a submitter preparing a MCAN for a new microorganism where the recipient and/or donor microorganisms have been reviewed in a prior submission to EPA may refer to information in the earlier submission and does not need to resubmit that information in the new MCAN.

2.Contract manufacturing

Comments:

One commenter (#39) had concerns about the applicability of the proposed regulations to contract manufacturing:

In the case where LTI performs contract fermentation and/or purification services for research products manufacturers, our customers may not be regulated by another outside agency. Some or all of such work may be covered by EPA regulations relating to R&D and production of these types of products. However, LTI believes that it would be both impractical and unduly burdensome for us to be required to file certifications and applications to EPA; this responsibility should fall to the commercial concern which intends to offer the products for general commercial sale. The contractors should be responsible for ascertaining what regulations apply to the construction of intergeneric plasmids and/or strains because only they will have detailed knowledge of the constructs which is necessary to determine appropriate compliance measures. Further, some of this information may be considered confidential and will likely not be available to LTI.

It is our understanding that the above interpretation is the intention of § 725.105(a)(2), and we request that EPA provide confirmation to that effect.

EPA response:

In response to the commenter (#39) who questioned how the proposed regulation would apply to contract manufacturing, EPA notes that the regulation at § 725.105 adopts the same approach to contract manufacturing of TSCA microorganisms as has been applied to contract manufacturing of other TSCA-regulated chemical substances.

In general, it is the manufacturer of a new microorganism who is required to submit the MCAN. However, where a person contracts with a manufacturer to produce an intergeneric microorganism and otherwise meets the conditions laid out in § 725.105(a)(2), the person who has contracted with the manufacturer will be required to submit the MCAN. In such a case, the

company that actually produces the intergeneric microorganism is considered a contract or toll manufacturer and the person with whom he or she has contracted (i.e., the contractor) will be considered a manufacturer for § 5 purposes. Although the toll manufacturer will not be required to file the MCAN under § 725.105(a)(2), since both persons involved in the transaction are manufacturers, both will be liable under TSCA § 15 if manufacture of the new microorganism commences before a MCAN has been submitted, or before the review period has expired.

In order for a manufacturer to be considered a toll manufacturer, the company must comply with all of the requirements in § 725.105(a)(2). Specifically, the manufacturer must produce or process the intergeneric microorganisms exclusively for the contractor, and the contractor must specify the identity of the microorganism and control both the total amount produced and the basic processes for making the microorganism.

Applying these requirements to the standard practices for microorganism manufacture, the term "produce or process" can include both the actual construction of the intergeneric microorganism, and/or growing large quantities of the microorganism (fermentation). Also, the term "specifies the identity of the microorganism" can include providing the intergeneric microorganism as an inoculum for fermentation, as well as providing detailed instructions for the actual construction of the intergeneric microorganism.

An "exclusive" relationship with the toll manufacturer means that the toll manufacturer does not sell or attempt to sell the microorganism to anyone else. As a result the contractor will also determine the total amount to be manufactured. Therefore, if more than one person orders the new microorganism, neither of these persons will have specified the total amount to be manufactured and neither is responsible for submitting the MCAN; instead, the person who actually manufactures the microorganism must submit the notice.

Although the contractor must specify the basic process for making the microorganism, under § 725.105(a)(2)(ii), the toll manufacturer may adjust the specified process as necessary to adapt the process to the toll manufacturer's own equipment. This situation would still fall within the scope of § 725.105 (a)(2), and the contractor would be considered a manufacturer, responsible for submitting the MCAN.

This provision does not apply to the person who simply orders a specific microorganism or a microorganism with certain properties from a manufacturer. In that case, the person ordering the microorganism would not be considered a manufacturer. Therefore, the actual manufacturer, who would not in this case be functioning as a toll manufacturer, would submit the MCAN.

EPA has included § 725.105 to address the situation in which one person decides to manufacture a specified amount of a microorganism using a particular process, but contracts with a toll manufacturer to actually produce the microorganism. In this case, EPA believes that the person contracting with the toll manufacturer will be the most knowledgeable party, and therefore he or she should be responsible for submitting the MCAN. EPA recognizes, however, that the actual

(toll) manufacturer will often have extensive information that would be useful to EPA in its review of the new microorganism. Therefore, EPA strongly encourages joint submissions (see § 725.25(e)) in this situation.

With regard to the specific scenario posed by the commenter (#39), assuming that the contractors for whom the commenter provides fermentation and/or purification services meet the requirements of § 725.105(a)(2), it would be the contractor who would be required to submit the MCAN. The commenter, however, would not be permitted to begin manufacture until a MCAN was submitted and reviewed, or until the review period had expired.

This discussion of contract manufacturing with respect to MCAN reporting would also be applicable to reporting and record keeping activities for commercial R&D activities for microorganisms. The microorganism R&D requirements, which are found in subpart E of part 725, are discussed in Unit IV. of this document.

3.Information requirements

Comments:

Six commenters (#8, 18, 25, 28, 33, 37) expressed concern about the information to be included in the MCAN, indicating that the requirements (§§ 725.155 and 725.160) were confusing and burdensome. Two commenters (#8, 18) noted that:

The prospective EPA 'review that considers all (emphasis added) the reasonable [sic] ascertainable information on...effects' can be interpreted as open ended and subject to challenge both by submitters and opponents to biotechnology. It would be better to review only that information likely to be pertinent to the change in the phenotype of the organism.

These commenters did not believe that testing should be required unless there is existing evidence for concern. One commenter (#25) noted that "there are a lack of acceptable and proven test protocols for many of these studies. Furthermore, the significance and interpretation of experimental results from many of these types of studies are poorly understood and have not been clearly established." Another commenter (#18) stated:

The information requested in the MCAN under genetic construction (2) and phenotypic and ecological characteristics (3) are so encompassing that it can be said that such information is not currently known for any microorganism. This section should be amended to read, 'where applicable to assessing the safety to humans and the environment, the following information should be submitted'. The information called for in this section will greatly help microbial ecologists, and indeed plant pathologists, in understanding the microorganism in question and its interaction with the environment, but have little or no relevance to safety or commercialization.

EPA response:

In response to the commenters who stated that the information required to be submitted in the MCAN was confusing, burdensome, and "open-ended and subject to challenge by submitters and opponents of biotechnology," EPA notes that both the proposed and final rule require submission only of the information that is explicitly required to be submitted by TSCA sections 5(b) and (d). In order to reduce the information that must be included in the MCAN, EPA would need to promulgate an exemption under § 5(h)(4). EPA does not currently possess the information necessary to determine that it would not need to review the data listed in §§ 725.155(c) through (h) and 725.160, to assess the wide variety of all potential new microorganisms in order to develop information requirements tiered to reflect potential microorganism characteristics.

The comments also reflected a substantial amount of confusion about what §§ 725.155(c) through (h) and 725.160 require. MCAN submitters are only required to submit the data specified in §§ 725.155(c) through (h) to the extent it is known to the submitter, or insofar as it is reasonably ascertainable, and to submit the data specified in § 725.160 to the extent the test data are in the submitter's possession or control. No requirements for specific tests are imposed by either section.

At § 720.3(p) "known to or reasonably ascertainable" is defined as "all information in a person's possession or control, plus all information that a reasonable person similarly situated might be expected to possess, control, or know." EPA has incorporated this definition in § 725.3 by reference.

Cost and burden are factors in determining whether information is known to or reasonably ascertainable by the submitter. EPA believes that a detailed definition of "reasonably ascertainable" may result in inequitable treatment of notice submitters. What would be a reasonable effort for one company under certain circumstances might be extremely burdensome for the same company under different circumstances, or for another company in the same situation. The amount and type of information meeting this definition will depend on the specific circumstances surrounding the development of the new microorganism. The nature of the recipient microorganism, the introduced genetic material, the conditions of use, the projected sales volume and profit, and the size of the company are factors in determining what information can reasonably be obtained.

EPA believes that "reasonably ascertainable" can be defined only on a case-by-case basis and that submitters must be responsible for deciding when and how to obtain required data, and when required information is not reasonably ascertainable. In most instances, data-gathering that is so costly as to preclude commercialization is not reasonable. To provide additional guidance to submitters, EPA will continue to make its "Points to Consider in the Preparation of Microorganism Submissions under TSCA" available to the public. Additionally, as noted in the

preamble to the proposed rule (59 FR 45530 (September, 1, 1994)), EPA recommends that potential submitters begin discussion with EPA staff early in the submission planning process so that EPA may provide guidance that is more specifically tailored to the MCAN submission for the submitter's microorganism.

EPA's MCAN requirements at §§ 725.155 and 725.160 were based entirely on TSCA §§ 5(b) and 5(d)(1). TSCA § 5(d)(1)(A) requires that the notice include the information described in subparagraphs (A), (B), (C), (D), (F), and (G) of § 8(a)(2) "insofar as known to the person submitting the notice or insofar as reasonably ascertainable." The requirements at § 725.155 correspond to these subparagraphs. Section 725.155(d) requires submission of microorganism identity information. This corresponds to TSCA § 8(a)(2)(A) which requires chemical identity and molecular structure information. For intergeneric microorganisms, the equivalent of chemical identity would include the taxonomic designations (genus and species) of the recipient microorganism and the donor(s) of the introduced genetic material as well as certain phenotypic and genotypic information. Many taxonomic designations at the species level define phenotypically and genotypically diverse groups of microorganisms. Therefore, supplemental information on phenotypic and genotypic traits is necessary to identify as precisely as possible a specific microorganism for Inventory listing.

Section 725.155(e) requires a description of byproducts. This corresponds to TSCA § 8(a)(2)(D) which requires a description of byproducts. The byproducts requirement is discussed further in this document in Unit IV.A.4. Section 725.155(f) requires information about total production volume. This corresponds to TSCA § 8(a)(2)(C) which requires information on the total amount of each substance manufactured or processed. Section 725.155(g) requires a description of categories of use and estimated production volume. This corresponds to TSCA § 8(a)(2)(B) which requires information on the categories of use and § 8(a)(2)(C) which requires reasonable estimates of the amount to be manufactured or processed for each category of use. Section 725.155(h) requires information on worker exposure and environmental release. This corresponds to TSCA § 8(a)(2)(F) which requires information on the number of individuals who will be exposed to the substance and duration of exposure.

Section 725.160(a) requires submission of "Test data on the new microorganism in the possession or control of the submitter." This corresponds to TSCA § 5(d)(1)(B) which requires that the notice include any test data in the possession or control of the submitter. Section 725.160(b) asks for "Other data concerning the health and environmental effects of the new microorganism that are known to or reasonably ascertainable by the submitter." This corresponds to § 5(d)(1)(C) which requires that the notice include a description of any other data concerning the environmental and health effects of the substance that are known to or reasonably ascertainable by the submitter.

The purpose of the MCAN is to supply EPA with information necessary to identify and list the

new microorganism on the TSCA Inventory and to determine whether the microorganism would pose an unreasonable risk to human health or the environment. In keeping with that objective, EPA has revised § 725.155(b) to explicitly include the statement that the submitter include all reasonably ascertainable information that will permit EPA to make a reasoned evaluation of the human health and environmental effects of the microorganism. If EPA finds that the information submitted in the MCAN is insufficient for EPA to complete its review, it may request the submitter to provide the additional information during the 90-day review period, or EPA will take action under TSCA § 5(e), where appropriate, to regulate the substance pending submission of the information.

The MCAN information requirements closely parallel those for PMNs and differ only to the extent necessary to accommodate the specific characteristics of microorganisms. The introductory paragraphs in § 725.155 have been revised to more closely parallel the introductory language in § 720.45, which contains the information requirements for the PMN. Therefore, the first two paragraphs of § 725.155 now read as follows:

(a) Each person who is required by this part to submit a MCAN must include the information specified in paragraphs (c) through (h) of this section, to the extent it is known to or reasonably ascertainable by that person. However, no person is required to include information which relates solely to exposure of humans or ecological populations outside of the United States.

(b) Each person should also submit, in writing, all other information known to or reasonably ascertainable by that person that would permit EPA to make a reasoned evaluation of the human health and environmental effects of the microorganism, or any microbial mixture or article, including information on its effects on humans, animals, plants, and other microorganisms, and in the environment. The information to be submitted under this subpart includes the information listed in paragraphs (c) through (h) of this section relating to the manufacture, processing, distribution in commerce, use, and disposal of the new microorganism.

EPA disagrees with the commenter (#18) who stated that information on genetic construction and phenotypic characteristics has little or no relevance to safety or commercialization. In addition to its use for identification and listing of microorganisms on the TSCA Inventory (as discussed in the proposed rule preamble, 59 FR 45551-51), EPA regards the information requested in § 725.155(d)(2) "Genetic construction of the new microorganism(s)" and (3) "Phenotypic and ecological characteristics" as essential to the development of its risk assessment for a new microorganism and pertinent to determining the change in the phenotype of the new microorganism relative to the parent microorganism. Information required in § 725.155(d)(2) would include a description of the genetic material introduced into the intergeneric microorganism and will help predict the likely behavior of the microorganism. For example, if genetic material encoding resistance to an antibiotic were included in the genetic construct, EPA scientists would evaluate the potential of the intergeneric microorganism to affect clinical use of the antibiotic.

EPA agrees with the commenters (#8, 18) who suggested that EPA review only information

pertinent to the change in phenotype of the microorganism. This is exactly what EPA does in its review; however, in order to compare the behavior of the new microorganism to that of the parent, EPA must have information on both the parent and the new microorganism. For example, in its reviews of PMNs for field tests of strains of *Rhizobium meliloti* genetically modified for enhanced nitrogen fixation, EPA compared the behavior of the new PMN strains to the behavior of naturally occurring rhizobia to determine whether the new PMN strains were behaving within a range of behaviors expected for naturally occurring rhizobia. EPA looked at field test data on nitrogen-fixation, survival, and competitiveness for the PMN strains but also needed the same information on naturally occurring strains to make its comparison. To date, EPA has found that in field tests, the PMN strains do behave within a range of behaviors expected for naturally occurring strains. This finding reduces uncertainty about the behavior of the PMN strains and has contributed to EPA's recent determinations that field tests of the PMN strains were not likely to present an unreasonable risk of injury to health and the environment.

With regard to the commenters (#8, 18) who believe that testing should only be required when there is existing evidence for concern, EPA notes the distinction between the requirements of TSCA § 5(d)(1)(B) and 5(d)(1)(C). As discussed above, TSCA § 5(d)(1)(B) instructs EPA to ask for test data, but this is limited to test data in "the possession or control of the submitter," whereas § 5(d)(1)(C) instructs EPA to ask for "a description of any other data concerning the environmental and health effects of such substance, insofar as known to the person making the notice or insofar as reasonably ascertainable." There are no requirements for specific tests to be conducted and the results submitted in the MCAN. However, if EPA finds that the information in the MCAN is insufficient and the microorganism may present an unreasonable risk or may be produced in substantial quantities which may result in substantial or significant exposure, under TSCA § 5(e) EPA may issue an order prohibiting or limiting production and specifying the type of information, including test data, that it needs to address its concerns. Alternatively, EPA may issue test rules under TSCA § 4 for specific chemical substances; however, to date EPA has not used TSCA § 4 for microorganisms.

EPA agrees with the commenter (#25) who noted the lack of acceptable test protocols for many microorganism studies. EPA believes that proven test protocols will become available as more experience is gained in microbial ecology. In the meantime, EPA also believes that while the interpretation of some experimental results may be based on partial understanding of environmental microbial processes, this does not mean that the information itself would not be useful, at least qualitatively, in EPA's review. Such information will allow EPA to develop the experience to devise validated testing procedures in the future.

4.Byproducts

Comments:

Three commenters (#28, 33, 35) expressed concern about the inclusion of byproducts in the § 725.3 definition of "manufacture, import, or process for commercial purposes" and the

corresponding information requirement at § 725.155(e). These commenters stated the following:

"This is a gray area since many cellular products are produced during a microbial fermentation. The vast majority of these compounds are never characterized. Furthermore, these compounds should be considered by EPA to be implicitly on the TSCA Inventory since they are produced by the unmodified microorganism. We are unclear why EPA has extended the definition to this category of substances. If there are distinct health and safety issues associated with byproducts they will be addressed during the MCAN review and are further subject to Section 8(e) reporting as appropriate. We recommend deleting this subpart in the final rule."

EPA response:

Commenters expressed concern that in the proposed rule EPA had "extended" the definition of "manufacture, import, or process for commercial purposes" to include byproducts. However, the language in the proposal is not an extension of the definition. The definition of "manufacture, import, or process for commercial purposes" in § 725.3 is adapted from the definition for "manufacture or import for commercial purposes" at § 720.3(r) which also includes byproducts. Further, as noted above in Unit III.A.3., TSCA § 5(d)(1)(A) requires EPA to collect such information. Note, however, that the information requirements for byproducts at § 725.155(e) is limited to information that is "known to or reasonably ascertainable" by the submitter. Thus, to the extent that the submitter chooses to specifically identify and characterize byproducts, that information must be submitted to EPA.

B.SNUR

EPA indicated that while it was not proposing SNURs for any microorganisms as part of the proposed rule, it was setting up the SNUR process for microorganisms as part of the regulatory text.

Comments:

Two commenters (#28, 33) had concerns about the SNUR process and stated "we caution EPA not to implement blanket SNURS. SNURS need to be communicated clearly so that manufacturers will understand their regulatory implication. Microbial products differ from traditional chemicals and there should be separate rulemaking for SNURS."

EPA response:

EPA does not intend to implement "blanket" SNURs for microorganisms. EPA intends to issue SNURs on a case-by-case basis, depending upon the need to evaluate the risk of some microorganisms when the environment of use is changed. To illustrate when a SNUR might be appropriate for microorganisms, as part of the preamble to the proposed rule EPA discussed an example of the kind of scenario that might trigger SNUR reporting in the future. This example

involved taking a microorganism commonly used for terrestrial icemaking and developing this microorganism for use in cloud seeding. EPA indicated that it believed that cloudseeding would present a significantly different exposure scenario and risk potential than terrestrial uses. Therefore, EPA might require SNUR reporting prior to the use of the live microorganism for cloudseeding. It was EPA's intent that this example give the public an idea as to the differences in use that might cause EPA to consider issuing a SNUR for microorganisms.

EPA agrees that SNURs need to be communicated clearly and for that reason has proposed to use expedited procedures only in instances where a TSCA § 5(e) consent order has been issued and the SNUR is consistent with the terms of the § 5(e) order. If the SNUR conditions differ from those of the § 5(e) order, or in those cases where no 5(e) order has been issued, EPA will use notice and comment procedures to explain the need for and specifics of the SNUR. The proposed Subpart L of Part 725 incorporated the Significant New Use Rule (SNUR) provisions from Part 721 with minor modifications to accommodate the specific characteristics of living microorganisms. EPA is promulgating Subpart L in the final rule with minor revisions, primarily to clarify the relationship of Subpart L to the other subparts in part 725. EPA has not yet proposed a SNUR for a specific microorganism.

C.TIERED EXEMPTION

EPA proposed, under TSCA § 5(h)(4), the Tier I and Tier II exemptions, which are an exemption from EPA review and an expedited EPA review, respectively, for specific microorganisms under certain use conditions at the general commercial use stage. The criteria defining eligibility for Tier I and Tier II exemptions address: (1) the recipient microorganism; (2) the introduced genetic material; and (3) physical containment to minimize the numbers of microorganisms emitted from the manufacturing facility.

Manufacturers meeting Tier I requirements will be completely exempt from review by EPA. EPA proposed that manufacturers submit a one-time certification statement to EPA 30 days prior to the first use at a facility of a microorganism eligible for a Tier I exemption. Subsequent uses of the same recipient microorganism would not require additional notification to EPA, so long as the manufacturer complied with all Tier I exemption conditions. Under the Tier I exemption requirements at § 725.424, manufacturers must identify the recipient microorganism and certify that the company is complying with the introduced genetic material criteria described in § 725.421 and the containment requirements described in § 725.422. Manufacturers must maintain documentation that the conditions of the exemption are met; see the record keeping provisions of §§ 725.65 and 725.450(d).

Like the Tier I exemption, the Tier II exemption also includes the requirements for the recipient microorganisms at § 725.420 and the introduced genetic material at § 725.421. However, EPA did not specify containment standards for microorganisms qualifying for the Tier II exemption. Therefore, submitters may choose containment conditions that vary from those listed at § 725.422, and these containment conditions would be subject to expedited EPA review. EPA

suggests that submitters use as guidance for appropriate containment conditions the standards set forth for Tier I procedures. Under the Tier II exemption, submitters must submit a Tier II exemption request which includes microorganism identity information (recipient microorganism must be listed in § 725.420), certification of compliance with the introduced genetic material criteria described in § 725.421, and process and containment information. EPA would approve or deny the exemption request within 45 days.

EPA received comments on issues related to the overall approach to the tiered exemption as well as the requirements for the three specific criteria defining eligibility for the tiered exemption. Comments on each of the specific criteria will be addressed separately following a discussion of more general comments.

1.General comments

Six commenters (#24, 28, 33, 34, 35, 39) generally supported the establishment of the tiered exemption. Five of these commenters (#28, 33, 34, 35, 39) made statements regarding administrative aspects of the tiered exemption. Four of these commenters (#28, 33, 35, 39) suggested alternative approaches for the tiered exemption.

a. Administrative issues

Comments:

Two commenters (#34, 35) indicated that it is "excessive and unwarranted" to require the submitter for a tiered exemption to certify that he/she is "...including with this submission all test data in my possession or control and a description of all other data known to or reasonably ascertainable by me..." as stated in § 725.25(b). One commenter (#39) stated that a 30-day review was not necessary and that companies working with organisms eligible for the Tier I exemption should simply be able to document their eligibility in their records. This commenter also suggested that EPA substitute a 10 day EPA review for the 30 day review specified at § 725.424(a)(4) for the certification. This commenter also stated that for the Tier I exemption, each institution should have its containment conditions approved only once for each eligible recipient, regardless of the introduced genetic material. Three commenters (#28, 33, 34) supported EPA's decision not to implement users' fees for the Tier I and Tier II exemptions.

EPA response:

For purposes of clarification, EPA is making several changes in this final rule to §725.424, Requirements for the Tier I Exemption, and §725.455, Information to be Included in the Tier II Exemption Request. EPA believes some of these modifications better elucidate the relationship between the certification statement at §725.25(b) and these tiered exemptions.

With regard to the first change, in the 1994 proposal, EPA inadvertently failed to reiterate at

§725.455 the requirements dealing with waste disposal and the certification statement, respectively, that were listed at §725.424 (b)(4) and §725.424(b)(5). Therefore, in the final rule for purposes of completeness and clarity, EPA lists at §725.455 the requirements dealing with waste disposal and the certification statement as §725.455(e) and §725.455(f) respectively. EPA does not believe reiteration of these requirements at §725.455 adds an additional burden, because submitters must under other Federal and local requirements have information about waste disposal of the microorganisms and other biological waste. Section 725.25(b) requires the certification statement and EPA reiterates this statement at §725.455(f) for the convenience of the submitter.

With regard to the second change, EPA modifies the certification statement at §725.424(b) and §725.455(f) to describe more clearly the relationship between the certification statement at §725.25(b) and the requirements at §725.424 and §725.455. The certification statement at §725.25(b) reads as follows:

I certify that to the best of my knowledge and belief: The company named in this submission intends to manufacture, import, or process for a commercial purpose, other than in small quantities solely for research and development, the microorganism identified in this submission. All information provided in this submission is complete and truthful as of the date of submission. I am including with this submission all test data in my possession or control and a description of all other data known to or reasonably ascertainable by me as required by 40 CFR 725.160 or 725.260.

Section 725.25 describes general administrative requirements, with §725.25(b) describing certification requirements in general for persons submitting to EPA. The first two sentences of this statement, therefore, where the company indicates that it intends to manufacture the microorganism identified in the submission and that all information is complete and truthful, are applicable to all submitters. However, the last sentence dealing with test data is not relevant to those persons certifying under §725.424 and §725.455. Rather, this sentence is relevant to persons preparing either the MCAN, who must meet the information requirements at §725.160, or meet the information requirements at §725.260. To clearly indicate the status of test data for those entities notifying for the tiered exemption, EPA has added a qualification to the requirement on certification at §725.424 and §725.455, indicating that certification of submission of test data is not required.

With regard to the third change, in order to emphasize the need to know the identity of the recipient microorganism, EPA has reordered §725.424 so that the requirement at §725.424(b)(3)(i) in the proposal becomes §725.424(b)(3) in the final rule. With this reordering, §725.424(b)(4) (which deals with waste disposal in the proposal) becomes §725.424(b)(5) in the final rule; §725.424(b)(5) (which in the proposal deals with the certification statement) becomes §725.424(b)(6) in the final rule. As revised, §725.424(b)(6) now reads as follows:

The certification statement required in §725.25(b). Certification of submission of test data is not

required for the Tier I exemption.

Similarly, §725.455(f) reads as follows:

The certification statement required in §725.25(b). Certification of submission of test data is not required for the Tier II exemption.

EPA agrees with commenter #39 that EPA review is not necessary for the Tier I exemption. Indeed, in making the § 5(h)(4) finding for the Tier I exemption (i.e., those microorganisms qualifying for the exemption under the specified conditions of use present no unreasonable risk), EPA makes the finding for the class and therefore obviates the need for a case-by-case evaluation of risk. Under the Tier I exemption, no data need be included in the submission for EPA to review. Under the Tier I exemption, EPA receives a one time certification that the submitter is complying with the criteria set out for the exemption so that EPA can be informed of which companies are taking the exemption. Once a manufacturer has sent in the certification required by §725.424, subsequent uses of the same recipient microorganism at the same or different facilities would not require additional certification as long as the manufacturer is continuing to comply with the introduced genetic material requirements of §725.421 and the containment requirements of §725.422.

While EPA does not believe that an EPA risk evaluation review is necessary each time microorganisms qualifying for the exemption at §725.424 are manufactured, EPA believes that it is appropriate for the Agency to be notified of which manufacturers are eligible for and utilizing the exemption so that EPA can keep track of the commercial use of new microorganisms, and respond to any public inquiries. However, EPA also has decided that since the purpose of the certification is to inform EPA that manufacturers are using the Tier I exemption, such notification is not needed 30 days in advance. Ten days in advance would be sufficient. Therefore, EPA has revised the requirement at §725.424(a)(4) to read: "The manufacturer or importer submits a certification described in paragraph (b) of this section to EPA at least 10 days before commencing initial manufacture or import of a new microorganism derived from a recipient microorganism listed in §725.420."

In this final rule, EPA has decided not to require payment of a fee for filing the Tier I certification or the Tier II exemption request. See the amendment to § 700.45(c).

b. <u>Alternative approaches</u>

<u>Comments</u>:

While generally supporting the tiered exemption, four commenters (#28, 33, 35, 39) suggested alternative approaches and modifications to EPA's three criteria. One commenter (#39) suggested that EPA develop a multilevel facility certification program utilizing certification levels which parallel the biosafety levels in the NIH Guidelines. This commenter suggested that "[o]nce

certified, a given facility would be eligible for automatic exemption from proposed EPA notification, certification, and filing requirements for activities using microorganisms at or below its certification level."

One commenter (#35) suggested that the Tier II exemption be extended, so that the requirements for recipient microorganism, introduced genetic material and containment could all be used as guidance and any of the three could be modified by the manufacturer. Two other commenters (#28, 33) felt that instead of requiring performance based standards for containment, EPA should utilize the Good Industrial Large-Scale Practices (GILSP) criteria developed by the Organization of Economic Cooperation and Development (OECD) found in the OECD document "Recombinant DNA Safety Considerations" (Ref. 2). The commenters believed that the OECD GILSP criteria place restrictions on the characteristics of the resulting new organisms but do not place additional requirements on containment.

EPA response:

EPA does not believe that the multilevel facility certification program suggested by one commenter (#39) would allow the Agency to appropriately discharge its responsibilities under TSCA § 5 at this time. TSCA addresses the manufacture and use of chemicals, including new microorganisms, and does not regulate facilities as such.

EPA does not believe that at this time it can develop a § 5(h)(4) exemption that would reduce both reporting requirements and EPA's review, and which would allow a submitter to modify the recipient microorganism and/or introduced genetic material as well as the containment criteria. As discussed in the preamble to the proposed rule (59 FR 45542 (September 1, 1994)), TSCA § 5(h)(4) provides that EPA may exempt by rule the manufacturer of any new chemical substance from all or part of the requirements of § 5, only if it determines that activities involving the substance will not present an unreasonable risk of injury to health or the environment. In developing § 5(h)(4) exemptions, EPA has found that the most suitable approach to making the necessary factual findings is to define criteria describing categories of substances that present low risk when used under specific conditions, and which therefore will support reducing the statutory reporting requirements.

To develop an exemption that did not involve EPA review, EPA had to prescribe conditions so that the finding that the microorganism "will not present an unreasonable risk" could be made *a priori*. Placing conditions on the three key aspects of the new microorganisms eligible for the exemption - i.e., the eligible recipient microorganisms, the introduced genetic material, and the conditions of use, ensures that the "no unreasonable risk" finding could be made for the microorganism without EPA review.

EPA's criteria for the introduced genetic material do not limit, except for certain toxin sequences, the types of characteristics that can be introduced into the recipient microorganism. Therefore, EPA believes it necessary to restrict such modifications to recipient microorganisms known to be

of low risk when used under the conditions prescribed by the criteria addressing containment. The requirements placed on the introduced genetic material primarily attempt to ensure that developers know the functions of the introduced genetic material and that it is poorly mobilizable. These requirements, in concert with the level of safety associated with the recipient microorganisms, ensure in part, that the resulting microorganisms will present low or negligible risk . For the Tier I exemption which involves no EPA review, the physical containment requirements provide the additional measure of risk reduction necessary for making the requisite § 5(h)(4) finding. EPA developed the Tier II exemption because EPA realized that in some circumstances the level of physical containment prescribed in the Tier I exemption would not be appropriate or sufficiently flexible, in light of the number of approaches to containment in fermentation facilities. For this reason, EPA developed the Tier II exemption for those submitters who needed more latitude in selecting their containment conditions. The list of eligible microorganisms and the criteria defining the introduced genetic material are the same as in the Tier I exemption. Thus, EPA need only evaluate whether the conditions of containment are appropriate for the microorganism being used to ensure that the finding of "will not present an unreasonable risk" required by § 5(h)(4) is made.

When there are no specific criteria which allow EPA to make an *a priori* finding regarding unreasonable risk, EPA must review the specific conditions surrounding the microorganism and its use in order to make the finding required under TSCA § 5. Therefore, the more changes in specified criteria that are allowed, the closer the reporting and review process resembles that followed for a MCAN. EPA believes that it is more appropriate for industry, and more understandable by the public, to have exemptions such as the Tier I and Tier II exemptions which are clearly delineated and represent an obvious reduction in burden relative to the MCAN.

With regard to the commenters' (#28, 33) suggestions that EPA use the OECD GILSP criteria, EPA's approach to the tiered exemption is consistent with the OECD GILSP. The three criteria which define the tiered exemption are based in large measure on the OECD GILSP. For the recipient microorganisms, EPA considered points also contained in GILSP when it selected candidates which have "a long history of safe use," "are not pathogens in the condition of use," and "are limited in their ability to survive in the environment and cause adverse environmental effects." Likewise, for the introduced DNA, the tiered exemption uses the same limitations as GILSP, namely well characterized, limited in size, and poorly mobilizable. Both the tiered exemption and GILSP also prescribe performance-based standards for containment.

Unlike GILSP, EPA did not place separate requirements on the resulting, new microorganism. EPA believed such requirements would be unnecessary because placing restrictions on the recipient microorganisms and the introduced genetic material that could be used would result in "new" microorganisms that meet the GILSP criteria, including the GILSP requirement that the resulting microorganism be non-pathogenic.

EPA's decision not to adopt the GILSP restrictions on the new microorganism was also based on its belief that the GILSP requirements that the resulting microorganism be non-pathogenic and as

safe as the host organism do not, as stated, provide the type of uniform, enforceable requirements necessary for a § 5(h)(4) exemption. EPA does not believe that the term "pathogenic" can be defined in a way that would avoid the necessity of a case-by-case review, which would defeat the purpose of the Tier I exemption and therefore would not be appropriate for the Tier I exemption. Subsequent to publishing the 1986 policy, EPA charged its Biotechnology Science Advisory Committee (BSAC) with developing a definition of "pathogen" which could be used in biotechnology regulations. This proved to be so difficult that the BSAC recommended that EPA abandon its attempts (Ref. 1A). Therefore, EPA did not utilize in the TSCA context an approach that codified a requirement to determine pathogenicity or non-pathogenicity. Instead, EPA has addressed that concept on a case-by-case basis in its selection of candidate microorganisms for the tiered exemption.

To develop a § 5(h)(4) exemption from EPA review, EPA needed more specific criteria than the guidance offered in GILSP. Because the characteristics of the recipient microorganism determine for the most part the parameters of the behavior of the new microorganism, EPA believes that the eligible recipient microorganism should be carefully defined. While EPA used some of the criteria described by OECD, such as history of safe industrial use, EPA believes that the greatest reduction in risk is obtained by listing as eligible for exemption specific microorganisms known to be low risk as EPA has done at § 725.420. EPA also spelled out

requirements for the introduced genetic material and conditions of containment, both to make the approach uniform and enforceable, and to ensure that the "will not present an unreasonable risk" standard of § 5(h)(4) is met.

2.<u>Recipient microorganisms</u>

Six criteria were used by EPA to determine eligibility of recipient microorganisms for the tiered exemption: (1) it should be possible to clearly identify and classify taxonomically the microorganisms using available genotypic and phenotypic information; (2) information should be available to evaluate the relationship of the eligible recipient microorganisms to any other closely related microorganisms which have a potential for adverse effects on human health or the environment; (3) there should be a history of safe commercial use for the microorganisms; (4) the commercial uses should indicate that the microorganisms' products might be subject to TSCA; (5) studies are available from which EPA can judge the potential for the microorganisms to cause adverse effects; and (6) studies are available from which EPA can judge the survival characteristics of the microorganisms in the environment. A risk assessment, which considered the six criteria, was prepared for each microorganism listed at § 725.420.

After completion of the risk assessments, a decision document was prepared for each microorganism listed at § 725.420. This decision document considered the potential risks of the recipient microorganisms as discussed in the risk assessments, and the potential risks from the resulting intergeneric microorganisms eligible for the Tier I and Tier II exemptions. To evaluate

the potential for an unreasonable risk of injury to health or the environment in making the § 5(h)(4) determination for these exemptions, EPA focused primarily on the characteristics of the recipient microorganisms. If the recipient microorganism was shown to have little or no potential for adverse effects, introduced genetic material meeting the specified criteria would not likely significantly increase the potential for adverse effects. As further assurance that risks would be low for the Tier I exemption, EPA is specifying containment conditions for minimizing the number of microorganisms released from the facility. For the Tier II exemption, EPA will review the containment conditions used. EPA found that when balanced against resource savings for society and expected product benefits, these exemptions will not present unreasonable risks. The full risk assessments and the decision documents for the recipient microorganisms listed at § 725.420 were included in the rulemaking docket and were made available for public review and comment.

EPA received 10 comments (#8, 12, 13, 18, 24, 28, 33, 34, 35, 39) on issues regarding the recipient microorganisms eligible for the tiered exemption. Four commenters (#8, 13, 18, 25) generally supported the criteria that recipient microorganisms must meet to be eligible candidates. Six commenters (#8, 12, 18, 28, 33, 35) had concerns about one or more of the six criteria. Seven commenters (#13, 24, 28, 33, 34, 35, 39) had comments about amending the list of eligible recipient microorganisms.

a.Criteria for eligibility

Comments:

Five commenters (#8, 18, 28, 33, 35) had concerns about the taxonomy criterion. Three commenters (#28, 33, 35) specifically asked that EPA recognize that microbial taxonomy is constantly changing. One of the three (#35) suggested that EPA consider the taxonomic designation used for eligible recipients to be inclusive of any taxa listed under historical or future revisions of the genera, as long as the microorganism lineage can be appropriately documented.

Two commenters (#8, 18) asked for clarification as to whether all six criteria must be met for a recipient to qualify for the exemption, since it appeared to those commenters that some of the ten candidate microorganisms did not meet all six criteria. These two commenters also were concerned about the history of safe use criterion. They indicated that, depending on the interpretation, a species might be considered historically safe but specific strains of that species may not have a history of safe use. These commenters sought clarification about the eligibility for all strains within a species qualifying for the exemption.

One commenter (#12) specifically indicated support for the criterion requiring the existence of studies concerning the potential for adverse effects and the survival characteristics of recipient microorganisms. This commenter questioned whether all ten recipient microorganisms were debilitated when used in fermentation processes. The commenter suggested that EPA should

consider an additional criterion, i.e., studies which document whether the ten candidates are debilitated.

EPA response:

EPA received no substantive comments either challenging EPA's approach to selecting recipient microorganisms for listing, or questioning the eligibility of the 10 microorganisms proposed as candidates for listing. Therefore, EPA has not made substantive changes to its approach to selecting recipient microorganisms. Section 725.420 continues to list the 10 microorganisms as eligible for use in the tiered exemption. However, in response to comments, EPA is providing additional explanation as to how the six criteria are used together to determine a microorganism's eligibility for listing at § 725.420.

One of the criteria used for selection of candidate microorganisms for the tiered exemption is clear identification and classification that enable the microorganisms to be assigned without confusion to an existing, easily recognized taxon. EPA believes that it is necessary to have an unambiguous and accurate description for the new microorganism. EPA recognizes that microbial taxonomy is constantly changing, and that modifications in taxonomic organization such as those recently seen for *Bacillus* and *Pseudomonas* may be expected in the future. EPA does not believe such reorganizations need affect the tiered exemption.

The risk assessments for the candidate microorganisms evaluated the hazards of the candidate microorganisms as they were appropriately designated taxonomically in 1994. If in the future the taxonomic designation of one of the recipient microorganisms changes, the microorganism evaluated in 1994 will still be exempt, even though it may possess a new name. For example, although the bacterium formerly designated as *Pseudomonas cepacia* is currently called *Burkholderia cepacia* due to advancements in analysis of genetic relatedness, the phenotypic characteristics of the microorganism have not changed, the available literature on the hazards associated with this microorganism has not changed, nor has the potential risk associated with its use, regardless of the new taxonomic designation.

EPA recognizes that a new taxonomic placement could affect the evaluation of the relationship of the microorganism to any other closely related microorganisms that may have a potential for adverse effects on human health or the environment, which is the second criterion used for selection of the recipient microorganisms for this tiered exemption. However, even if a change in taxonomic designation for the recipient microorganisms places them in taxa containing pathogens, the characteristics of the recipient microorganisms will remain unchanged.

EPA does not agree with the commenter (#35) who suggested that taxonomic designation used for eligible recipients be inclusive of any taxa listed under historical or future revisions of the genera. The risk assessments were conducted on the species as they were designated at the time of the assessments in 1994. Microorganisms that were historically subsumed under one species name, but were not considered members of that species in 1994 when the risk assessments were

prepared, would not have been reviewed by EPA during the preparation of the assessments. EPA evaluated all members of species of which each eligible microorganism belonged as of 1994. Thus, EPA will not accept historical taxonomic designations for the microorganisms listed at §725.420.

On the other hand, if a taxonomic change does occur in the future for any of the recipient microorganisms listed at §725.420, companies will need to demonstrate and document in their records that the microorganism being used under the tiered exemption would have been classified using a name listed in §725.420 at the time that EPA completed the risk assessment for that microorganism. For example, if in the future the name is changed for one of the ten microorganisms currently listed in §725.420, submitters would need to document that their microorganisms would have been classified in 1994 under the name listed in §725.420. Submitters should consult the "Identification and Taxonomy" section of the individual Risk Assessments for a detailed discussion of the taxonomy of each of the microorganisms listed in §725.420.

EPA wishes to clarify that a microorganism which is a candidate for exemption would not necessarily be eliminated from consideration if it did not meet all six criteria. In preparing the list of exemption candidates included in the final rule at §725.420, EPA considered all of the criteria together to determine whether, on balance, the facts supported a determination that the candidates would not present an unreasonable risk of injury to human health or the environment. For example, not all strains of the species *E. coli* were proposed for exemption because some strains are pathogens and therefore, the species as a whole did not meet the criteria. On the other hand, the strain *E. coli* K-12 did meet the criteria and was retained as a candidate for the exemption. For other cases, mitigating factors, such as the usual fermentation techniques known to be used with *A. oryzae*, limited concern for *A. oryzae* under those use conditions. Even though the risk assessment recognized the potential for toxin production by some members of the *A. oryzae* species, procedures generally employed in commercial production were found to minimize the concerns. When taken together, the criteria applied to the candidates proposed by EPA during the evaluation led to a conclusion that the candidate microorganisms warranted exemption. EPA intended that the ten risk assessments, in conjunction with the proposed rule, would illustrate how microorganisms with diverse characteristics could qualify for the exemption. Commenters should consult EPA’s final decision documents and risk assessments for additional guidance on the way the Agency used the six criteria to evaluate potential risk.

The history of safe use criterion was a major consideration in the selection of candidates for the proposed tiered exemptions. EPA chose species or strains that had significant commercial utility and with which there had been considerable commercial experience without incidence of adverse effects on human health or the environment; the history of safe use mitigated uncertainty about the potential risks posed by the microorganisms under conditions of commercial fermentation. The microorganisms’ history of safe use was balanced against the other five criteria in the risk assessments conducted on these ten recipient microorganisms. In some cases the commercial experience was limited to a specific strain within a species and did not apply to all strains within

that species. However, the risk assessments, which considered the potential adverse effects to human health and the environment along with the potential worker and environmental exposure, were conducted for the most part on the entire species of which the candidate microorganisms were a member. The only exception was the risk assessment for *E. coli* K-12 which considered only this specific strain of the species. The species *E. coli* did not meet the low risk test posed by the six criteria, and therefore the species was not proposed for the tiered exemption.

EPA wishes to further clarify its thinking on two of the six criteria used to determine eligibility of recipient microorganisms for the tiered exemption: the availability of studies which can be used to evaluate the potential for the microorganism to cause adverse effects on human health and the environment; and the availability of studies which can be used to evaluate the survival characteristics of the microorganism in the environment. EPA is not suggesting that a manufacturer proposing a microorganism for addition to the list at § 725.420 be required to perform hypothesis testing to ascertain the potential for adverse effects on human health or the environment, or the survival characteristics in all environmental media. Rather, EPA is emphasizing that for a candidate microorganism for inclusion on the list at § 725.420, enough needs to be known concerning the environmental behavior of the recipient microorganism to make a judgement concerning its potential risk to the environment. Such knowledge might be gathered through specific testing of the recipient microorganism, or through available scientific literature on the same species or strain. Knowledge of the survival characteristics and the potential for members of the species to cause adverse effects on human health or the environment is necessary in order for EPA to prepare the risk assessment needed to evaluate the eligibility of the recipient microorganism for the tiered exemption. This information, when used in conjunction with the other criteria at §§ 725.421 and 725.422, is important to the determination that the recipient microorganism will not present an unreasonable risk of injury to human health and the environment.

There is no criterion requiring candidate recipient microorganisms on the list at §725.420 to be debilitated. Microorganisms used in industrial fermentation are often debilitated, either intentionally for proprietary reasons or because they have become acclimated to the optimal growth conditions of the fermentation systems. Therefore, these microorganisms would, most likely, exhibit reduced survival in the environment relative to environmental isolates of the same species. EPA considered the potential for survival of the microorganisms in the environment in the risk assessments conducted in support of the tiered exemptions and will consider the issue in assessing future candidates.

b. <u>Amending the list of candidates</u>

<u>Comments</u>:

Six commenters (#24, 28, 33, 34, 35, 39) generally supported provisions to allow additional recipient microorganisms to be added to the candidate list at § 725.420. Two commenters (#34, 35) supported EPA's petition provisions at proposed § 725.67 which allow the public to propose

additional candidates. Three commenters (#28, 33, 35) indicated a desire to propose additional candidates and sought guidance on this process. Two commenters (#24, 39) suggested specific candidates to be added to the list at § 725.420.

Two commenters (#13, 35) had questions about specific candidate microorganisms. One commenter (#35) asked EPA to clarify whether the microorganism *Bacillus amyloliquefaciens* would be considered a variant of the listed candidate *Bacillus subtilis* and thus eligible for the tiered exemption. Another commenter (#13) asked that EPA add *Pseudomonas fluorescens* as an additional recipient microorganism eligible for the tiered exemption. This commenter expressed the opinion that the containment they employed and the limitations on the genetic material they introduced into certain of their products would make their products eligible for the Tier I exemption if *P. fluorescens* was listed as a recipient microorganism.

EPA response:

Petition process. EPA supports the concept of adding additional candidates to the list at § 725.420 of microorganisms eligible for the tiered exemption. As EPA gains additional experience reviewing intergeneric microorganisms, it will continue to use its authority under TSCA § 5(h)(4) to develop exemptions for low risk microorganisms. However, the petition process at § 725.67 can also be used by the public to propose candidates for inclusion on the list at § 725.420, and to provide the appropriate supporting information. The risk assessments and decision documents included in the docket intended to serve as models to the public of the level of information necessary to support petitions of eligibility for listing microorganisms at § 725.420. As a general matter, however, EPA expects that petitions to add specific recipient microorganisms to the list at § 725.420 will ideally be preceded by several MCANs which would provide relevant experience with, and information on, the microorganism. This accumulated experience should provide EPA with sufficient information following receipt of a petition to determine whether the recipient microorganism should be listed as a candidate for the tiered exemption.

The petition process was designed to be used by anyone seeking to apply for a § 5(h)(4) exemption from full MCAN reporting under TSCA § 5. The provisions at § 725.67(a)(2) indicate that petitioners should provide information to show that activities under the requested exemption will not present unreasonable risk of injury to health or the environment. This would include a description of the benefits of the new microorganism and the economic consequences of granting or denying the exemption. EPA has revised the regulatory text for the petition process at § 725.67 generally to clarify that the information included in a petition will mirror the information requirements for the provision for which the exemption is being sought. This revised regulatory text reads as follows:

(3) Specific requirements. In addition to the requirements of paragraph (a)(2) of this section, the specific information requirements of the relevant subpart under which the exemption is sought should be met.

(i) Exemption from MCAN reporting under subpart D. Information requirements are set forth in §§ 725.155 and 725.160.

(ii) Exemption from TERA reporting under subpart E. Information requirements are set forth in §§ 725.255 and 725.260.

(iii) Listing a recipient microorganism as eligible for exemption under subpart G. Information regarding the following criteria should be addressed in an application to list a recipient microorganism under § 725.420:

(A) Identification and classification of the microorganism using available genotypic and phenotypic information;

(B) Information to evaluate the relationship of the microorganism to any other closely related microorganisms which have a potential for adverse effects on human health or the environment;

(C) A history of safe commercial use for the microorganism;

(D) Commercial uses indicating that the microorganism products might be subject to TSCA;

(E) Studies which indicate the potential for the microorganism to cause adverse effects to human health or the environment; and

(F) Studies which indicate the survival characteristics of the microorganism in the environment.

Specifically, with regard to the tiered exemption, EPA has indicated at § 725.67(a)(3)(iii) that when applying to list a recipient microorganism for the tiered exemption under § 725.420, persons should include information addressing the six criteria, discussed above in Unit III.C.2.a., which EPA used to evaluate microorganisms for listing. Persons who wish to propose other microorganisms as candidates for exemption under subpart G of these regulations are encouraged to use the risk assessments and decision documents in the docket as models to develop their petitions under § 725.67(a)(3)(iii), as well as to consult with EPA regarding the preparation of their petitions.

Bacillus amyloliquefaciens. EPA wishes to clarify that the tiered exemption applies only to the recipient microorganisms currently listed in § 725.420. EPA does not believe that *Bacillus amyloliquefaciens* should be subsumed under the exemption for *Bacillus subtilis*. *B. amyloliquefaciens* may have been considered a variant of *B. subtilis* in the past; however, by the time the risk assessment for *B. subtilis* was developed in 1994 and when this Response to Comments document was written, *B. amyloliquefaciens* had been given separate species status from *B. subtilis* (Ref. 3). Therefore, *B. amyloliquefaciens* is not synonymous with *B. subtilis*, and EPA cannot include the former under the exemption for the latter. The risk assessment for *B.*

subtilis does not extend to *B. amyloliquefaciens*, because it was not evaluated in the *B. subtilis* assessment. However, if the commenter believes that *B. amyloliquefaciens* warrants an exemption based on the criteria EPA used to evaluate *B. subtilis*, EPA invites the commenter, or anyone else, to examine the risk assessment prepared for exemption of *B. subtilis*, and utilizing this assessment as a model for the issues important for eligibility, provide information to EPA supporting a separate listing for *B. amyloliquefaciens* as a recipient microorganism under § 725.420.

Pseudomonas fluorescens. A commenter (#13) proposed the species *Pseudomonas fluorescens* as a candidate for the tiered exemption in comments submitted on the proposed rule. Therefore, EPA is responding to this item as a rule comment and not as a formal petition. EPA's response also further illustrates the approach to considering eligible candidates. As discussed above in this section, commenters are referred to the process outlined in §725.67(a)(3)(iii) of the final rule, as well as the risk assessments and decision documents in the docket. The risk assessment is conducted on the naturally occurring recipient microorganism. Once a recipient microorganism has been determined to present a low risk, EPA further determines that use of

introduced genetic material meeting the criteria at §725.421, and containment conditions meeting the criteria at §725.422 for Tier I or reviewed by EPA for Tier II, will provide additional assurance that the risks will remain low.

EPA does not agree with the commenter (#13) who suggested that the species *Pseudomonas fluorescens* is an appropriate candidate for the tiered exemption at this time. As discussed above in this section, EPA selected the microorganisms proposed for this exemption by balancing together six criteria to estimate the overall risk of the recipient microorganisms. Although the proposed candidate microorganisms are not required to meet each of the six criteria, on balance, the facts must support the finding that under the conditions of the exemption, the microorganism will not present an unreasonable risk of injury to health or the environment. When EPA considered the information referenced by the commenter (#13) as well as information available from risk assessments EPA has prepared during PMN reviews involving strains of *P. fluorescens*, EPA did not agree that the species *P. fluorescens* on balance met the six criteria.

The taxonomy of the genus *Pseudomonas* is quite complicated and is constantly evolving with further research in phylogenetic relatedness. According to Palleroni (Ref. 4), the species *P. fluorescens* is quite heterogeneous. Within the fluorescent pseudomonad supercluster, there are five separate biovars of *P. fluorescens*, I-V, (biovars I-III formerly Biotypes A-C; biovars IV-V, formerly Biotypes F-G) two biovars (A and B) of *P. putida*, *P. chlororaphis* and *P. aureofaciens* (formerly *P. fluorescens* Biotypes E and D, which are now considered synonymous), *P. lundensis* and *P. fragi* (Ref. 5). According to Molin and Ternstrom (Ref. 6), "there are obvious problems in drawing lines of demarcation between the different biovars of the *P. fluorescens-P. putida* complex, as well as between the different subclusters of the *P. fragi* complex". On a phenotypic basis, there exist various continua within the two complexes (Ref. 6). Likewise, Hildebrand, et al., (Ref. 7) also showed the presence of continua based on DNA-DNA hybridization studies.

In addition, the species *P. fluorescens* poses concern relative to the fifth criterion - the ability of the microorganism to adversely affect human health or the environment. Recently, members of the species *P. marginalis*, which are plant pathogens, were incorporated into the species *P. fluorescens* Biovar II on the basis of DNA homology (Ref. 5). There are a number of oxidase-positive fluorescent pseudomonads that are capable of soft-rotting a number of plants such as carrots, cabbage, celery, onion, cucumber, cauliflower, lettuce, potato, chicory, fennel, leek, garlic, and hyacinth (Ref. 9). Currently, soft-rotting strains of fluorescent pseudomonads cannot be distinguished from nonsoft-rotting, oxidase-positive strains that are not plant pathogens except for their ability to cause soft rot. However, the common test of potato tuber maceration as an indication of soft-rot producing ability has been shown to be unreliable as an indicator of ability to cause soft rot in other plants (Ref. 9). Saprophytic and phytopathogenic strains are scattered throughout Biovar II (Ref. 10).

In addition, the history of safe use criterion has not been met. Although a particular company may have a few years of experience with a particular strain, a single strain is not representative of the entire species. The tiered exemption was not designed primarily for particular proprietary strains, but was intended for species that were widely available, widely used strains with decades of commercial use in the fermentation industry. The tiered exemption is not intended to be a replacement for a MCAN review of particular strains from a particular company.

In summary, EPA's conclusion after review of the information supplied by commenter #13, and that referenced above is that the species *P. fluorescens* is not eligible for listing as a recipient microorganism under § 725.420 at this time for the following reasons: its confusing taxonomic status; its lack of history of safe commercial use; and the potential of some strains currently classified as *P. fluorescens* to cause adverse effects on human health and the environment, particularly in relation to plant pathogenicity. EPA's review does not represent a full consideration of the eligibility for exemption of the species *P. fluorescens*, however, because sufficient information to perform an evaluation has not been submitted to the Agency.

3.Introduced genetic material

For the introduced genetic material, EPA identified four requirements in § 725.421 which must be met to qualify for the Tier I or Tier II exemptions: the introduced genetic material must be (a) limited in size, (b) well characterized, (c) poorly mobilizable, and (d) free of certain sequences. Comments received on these four conditions will be discussed in separate sections below.

Because EPA has determined that a definition of "new" based on intergeneric microorganisms is appropriate, EPA has provided the responses to comments on the criteria for the introduced genetic material within the context of the intergeneric scope. The terms used in the regulatory text for the tiered exemption should also be interpreted in this context. While the term "introduced genetic material" may apply scientifically to both intergeneric and intrageneric genetic material, only the intergeneric portions of the introduced genetic material must meet the requirements at § 725.421. Therefore, all terms associated with requirements in § 725.421 refer

solely to the introduced genetic material which is derived from an organism classified in a different genus from the recipient microorganism.

a.Limited in size

The requirements for the "limited in size" criterion are set forth at § 725.421(a) which states that the introduced genetic material must consist only of the following: (1) The structural gene(s) of interest; (2) The regulatory sequences permitting the expression of solely the gene(s) of interest; (3) Associated nucleotide sequences needed to move genetic material, including linkers, homopolymers, adaptors, transposons, insertion sequences, and restriction enzyme sites; (4) Nucleotide sequences needed for vector transfer; and (5) Nucleotide sequences needed for vector maintenance. EPA discussed its rationale supporting the limited in size criterion in the preamble to the proposed rule (59 FR 45547 (September 1, 1994)).

Comments:

EPA received 5 comments (#25, 28, 33, 34, and 35) on its "limited in size" requirements. These comments were generally concerned with clarifying which vector sequences would meet the criterion, including the status of certain sequences found in well-known, frequently used plasmids.

Five commenters (#25, 28, 33, 34, and 35) sought clarification as to which vector sequences would meet the "limited in size" criterion. They were concerned about the status of vector sequences which were derived from a microorganism belonging to a different genus than the "new" microorganism subject to TSCA, which were needed to transfer and/or maintain the introduced genetic material in "intermediate" microorganisms other than the final TSCA-subject microorganism. These vector sequences, although necessary in the "intermediate" host microorganism, would not be needed or expressed in the final microorganism intended to be used under the tiered exemption. The commenters questioned whether such intermediate host vector sequences would meet the limited in size criterion. Two methods were proposed by commenters to address intermediate host vector sequences: (1) the criteria at § 725.421 should be interpreted to include host vector sequences expressed in intermediate hosts and necessary for transfer and/or maintenance in those hosts and thus these sequences would be considered necessary for vector maintenance or transfer; and/or (2) a list of low risk vectors that could be used with the recipient microorganisms could be added to § 725.420. Such a list could be based on Appendix E of the NIH Guidelines.

Two commenters (#28 and 33) raised one additional vector-related question. Some well-characterized and commonly-used vectors such as pBR322 have sequences (antibiotic resistances, and others) that do not *prima facie* meet the criteria at § 725.421. Moreover, these intermediate vector sequences may be expressed in the TSCA-subject microorganism. For example, the pUC series of plasmids is approximately 1.3 kb smaller than pBR322, yet pUC plasmids are transferred

and maintained as well as pBR322 in some bacteria. The commenters asked whether this meant that pBR322 would not meet the criteria at § 725.421, since pBR322 contains sequences beyond those essential for transfer and maintenance. These commenters suggested that either the limited in size criteria be expanded to allow for expressed intermediate vector sequences, or a list of vectors meeting the limited in size requirement at § 725.421(a) be provided.

EPA response:

Concerning vector sequences necessary for transfer and/or maintenance in an intermediate host but not expressed in the TSCA-subject microorganism, the requirement for the introduced genetic material at § 725.421(a)(3) allows "Associated nucleotide sequences needed to move genetic material, including linkers, homopolymers, adaptors, transposons, insertion sequences, and restriction enzyme sites". EPA interprets this requirement to allow the introduced genetic material to contain vector material needed for maintenance in and/or transfer to intermediate

hosts, provided this vector material is not expressed in the intergeneric microorganism that will be manufactured under the tiered exemption. Such nonexpressed vector material should not change the behavior of the TSCA-subject microorganism.

The additional issue raised by commenters concerned vector sequences which are expressed in the new microorganisms and are part of recognized host-vector systems. The NIH Recombinant DNA Advisory Committee has, over the years, developed and been guided by concepts similar to the criteria EPA used to evaluate the risks of the introduced genetic material under § 725.421. The NIH Guidelines Appendix E list of certified host-vector systems and Appendix I list of biologically contained host-vector systems are examples of the use of these concepts to qualify risk (Ref. 11). Specific combinations of hosts, plasmids, and phages listed in these Appendices are exempt from the NIH Guidelines. The rationale underlying this exemption is based on the high degree of biological containment offered by these host-vector combinations. For example, for host-vector systems in which the vector is a plasmid, the NIH Guidelines specifies that the frequency of mobilization of plasmids in Appendices E and I is no more than 10^{8} . Because the EPA criteria are based on the concepts originated by the NIH RAC, EPA believes that pBR322, and other plasmid and phage vectors listed in Appendices E and I of the NIH Guidelines would meet the introduced genetic material criteria at § 725.421, including the limited in size criteria (Refs. 12, 13, 14). The specific vectors appropriate for use with the EPA candidate microorganisms *Bacillus subtilis*, *Escherichia coli K-12*, and *Saccharomyces cerevisiae* are listed in Appendices E and I of the NIH Guidelines (Ref. 11). EPA anticipates that when additional microorganisms are added to the EPA recipient microorganism list at § 725.420, the associated vectors listed by NIH as part of host-vector pairs for these recipients would likely be considered to meet the criteria at § 725.421.

b.Well characterized

The requirements for the "well characterized" criterion are set forth at § 725.421(b) which states

that well characterized means that the following have been determined for the introduced genetic material: (1) The function of all of the products expressed from the structural gene(s); (2) The function of sequences that participate in the regulation of expression of the structural gene(s); and (3) The presence or absence of associated nucleotide sequences where associated nucleotide sequences are defined as those "needed to move genetic material, including linkers, homopolymers, adaptors, transposons, insertion sequences, and restriction enzyme sites." EPA discussed its rationale supporting the well characterized criterion in the preamble to the proposed rule (59 FR 45547 (September 1, 1994)).

Comments:

EPA received six comments (#8, 18, 28, 33, 34, 35) on its "well characterized" criterion. Four commenters (#8, 18, 28, 33) noted that it may be difficult to know the functions of all products expressed by the structural genes. Two of these commenters (#8, 18) recommended replacing the term "function" with "nature" in the requirement at § 725.421(b)(1).

Three commenters (#28, 33, 35) requested further guidance from EPA on how to address open reading frames (ORFs) present in the introduced genetic material, since multiple reading frames had been discussed in the preamble of the proposal. Two of these commenters (#28, 33) stated that "[a]ll DNA sequences have the potential for alternate ORFs. Some of these ORFs may be of sufficient size to code for proteins, but many of these would be short ORFs with insufficient coding capacity. A clearer definition of what constitutes an ORF is required."

Two commenters (#28, 33) were concerned that the requirement at § 725.421(b)(2) could be difficult to meet for some upstream activator sequences, depending on how EPA interprets the term "function". These commenters pointed out that the mode of action of upstream activator sequences may not have been elucidated, although they are necessary for enhanced expression and are well-described in the literature. In this case, the commenters wanted to know what information is needed to meet the criteria at § 725.421(b).

One commenter (#34) questioned whether EPA would require complete genomic sequencing of the final construct to meet the well characterized definition.

EPA Response:

With regard to commenters' concerns about the level of characterization necessary to qualify for the criteria at § 725.421, EPA's intent in developing the well characterized criterion was to ensure that the functions introduced with the genetic material were sufficiently understood to predict the likely behavior of the resulting microorganism. To this end, manufacturers claiming eligibility for the exemption must characterize the introduced genetic material and the functions of the products of that introduced genetic material to the degree necessary to predict the likely behavior of the resulting microorganism. Because EPA defined a new microorganism as an intergeneric microorganism, it is the predicted effect of the intergeneric sequences on the phenotype of the

recipient microorganism that must be evaluated. These assessments must be performed in order to reduce the uncertainty that the intergeneric microorganism will express unanticipated novel traits beyond those intended by the introduction of the intergeneric sequences.

As required under TSCA § 5(d)(1)(a), these assessments should be performed using information that is known to or reasonably ascertainable by the person utilizing the exemption. (See Unit III.A. of this document for a discussion of "reasonably ascertainable"). EPA will not specify the techniques which should be used to show that no unanticipated novel traits are likely to be expressed from the introduced intergeneric genetic material but expects those utilizing the tiered exemption to use their best scientific judgement and to maintain good records describing how they arrived at their conclusions.

As noted earlier in this document (Unit III.A.), EPA will not provide a single standard defining what is "reasonably ascertainable" information. It should be noted, however, that the criteria at §§ 725.420 through 725.422 establish conditions of eligibility for exemption from the

MCAN reporting requirements. Should the introduced genetic material not meet the criteria laid out at § 725.421, manufacturers must submit a MCAN to EPA 90 days prior to first commercial manufacture.

With regard to the functions of products expressed by introduced structural genes, manufacturers could rely, for example, on peer-reviewed literature on products of structural genes and/or the results of protein expression assays to characterize the function(s) of a gene product. As manufacturers need only obtain information that is reasonably ascertainable, they would not be expected to perform batteries of tests to attempt to discover as yet unidentified functions of protein products. The peer reviewed article of Baldwin et al (Ref. 15) and its associated references (see in particular Figure 1), for example, illustrate the level of understanding which is considered to be "reasonably ascertainable" and appropriate to showing that the functions of the *lux* genes of *Vibrio fischeri* are known.

EPA believes that most manufacturers wishing to utilize the exemption would be able to meet the requirements at § 725.421. There is an economic incentive for manufacturers to fully characterize their microorganism prior to scale up to large volume fermentations. Large-scale fermentations utilize substrates which in aggregate can be costly and manufacturers are likely to guard against the possibility that their microorganism would display unexpected behaviors during fermentations. They are also likely to ensure that the introduced genetic material expresses the desired product at desired levels.

EPA does not intend the criterion at § 725.421(b) to imply that knowledge of the exact amino acid or protein sequence is required, nor that sequencing of the introduced genetic material is required. However, as noted by one commenter (#34), sequence information may be available on specific regions because the information was acquired in intermediate steps and is integral to development of the product. Such information would be considered to be "reasonably

ascertainable", under TSCA § 5(d)(1)(a).

The term "nature" has not been substituted for "function" at § 725.421(b) as suggested by two commenters (#8 and 18), because this change does not lead to any greater clarity in the regulatory text and the commenters offered no explanation as to how this change would address their concerns.

With regard to commenters' questions about ORFs and their relationship to the well characterized criterion, EPA noted in the preamble of the proposed rule that the definition of well characterized would include knowing whether multiple reading frames exist within the introduced genetic material (59 FR 45547 (September 1, 1994)). Multiple reading frames are a type of ORF. Manufacturers must ensure, by evaluating ORFs and multiple reading frames, that unanticipated novel traits are not expressed by the intergeneric microorganism. ORFs must be assessed to determine whether a product other than the anticipated, desired product is likely to be expressed from the introduced genetic material and to predict whether such a product(s), if expressed, would have an effect on the phenotype of the intergeneric microorganism.

Examples of how manufacturers can determine whether ORFs are present in the introduced genetic material include an analysis of nucleic acid sequences for ORF attributes. Manufacturers could examine a sequence for features such as start codons, a size sufficient to encode a protein (small sequences below 200 base pairs are unlikely to encode a protein) and ribosomal binding sites. To determine whether an ORF could affect the behavior of the microorganism, manufacturers could search for similarity to entries in computerized nucleic acid or protein sequence databases.

With regard to commenters' questions about upstream activator sequences (UASs), UASs are found in eukaryotes and perform a function similar to a promoter in that they are thought to aid in the binding of transcription factors to promoter regions. However, unlike a promoter, upstream activator sequences can be located at varying distances upstream from the start site for transcription and still result in an increased activity of promoters in their vicinity. UASs control the activity of other regions of genetic material that act as recognition sites for the initiation of protein synthesis, and do not encode proteins or functional RNA molecules. UASs, thus, are regulatory regions which solely control the activity of other regions (promoters) that act as recognition sites for the initiation of protein synthesis.

In determining the status of UASs with regard to the exemption at § 725.421, manufacturers must first consider whether introduction of the UAS would create an intergeneric microorganism. Because EPA is retaining the interpretation that an intergeneric microorganism is a new microorganism, a UAS(s) that is intrageneric would not trigger § 5 requirements. For a UAS isolated from an organism in a different genus from the recipient microorganism, manufacturers must determine whether their UAS meets the requirements in the definitions at § 725.3 for "non-coding regulatory region" and for "well-characterized". Modifications to these definitions are discussed in Unit II.D. of this document. Microorganisms developed through the introduction of

only UAS genetic material that is isolated from an organism in a different genus and that meets the above noted definitions at § 725.3 are excluded from the definition of "intergeneric microorganism" and therefore are not subject to the reporting requirements of TSCA § 5.

Manufacturers who wish to utilize the tiered exemption for microorganisms that contain both a UAS(s) and other genetic material isolated from an organism(s) in a different genus than the recipient, to meet the exemption requirements must: (1) ensure that the UAS meets the definitions of "non-coding regulatory region" and "well-characterized" at § 725.3; (2) ensure that the other introduced genetic material meets the requirements at § 725.421(b); and (3) ensure that the other requirements of the Tier I or Tier II exemption are met.

c.Poorly mobilizable

The requirements for the poorly mobilizable criterion are set forth at § 725.421(c) which states that the probability that the introduced material would be transferred to other

microorganisms must be low, with a frequency of transfer of less than 10^{8} transfer events per recipient. EPA discussed its rationale supporting the poorly mobilizable criterion in the preamble to the proposed rule (59 FR 45547-48 (September 1, 1994)).

Comments:

EPA received six comments (#8, 18, 28, 33, 34, and 35) on its "poorly mobilizable" criterion. Four of the commenters (#28, 33, 34, and 35) agreed that some limitations should be put on the mobility of introduced genetic material, and two commenters (#8 and 18) agreed that the 10^{-8} criterion was appropriate.

Although two commenters (#8 and 18) stated that the 10^{-8} criterion is reasonable and prudent for sequences that pose significant risk, one of these commenters (#18) argued that a higher mobility standard should be acceptable in cases where the nature of the introduced sequences indicates low to negligible risk. The other commenter (#8) noted that the introduced "property is the significant issue, not the transfer frequency."

Three commenters (#28, 33, and 35) noted that many factors affect the frequency of transfer by conjugation, transformation, and transduction: factors associated with the recipient microorganism such as restriction enzyme systems, acceptable integration sites, compatible recombination and replication functions, competency for transformation, phage receptors for transduction, and compatibility for conjugation; factors associated with the experimental conditions under which transfer rates are to be measured such as presence of nucleases and other environmental conditions. The commenters requested clarification on the conditions under which the 10^{-8} criterion should be measured.

Four commenters (#28, 33, 34, and 35) stated that microorganisms whose introduced genetic

material is stably integrated into the chromosome and do not have functional transposons should qualify for the 10^{-8} criterion. They also requested clarification of the following EPA statement in the preamble to the proposed rule: "In instances where introduced genetic material is located on the chromosome, steps can be taken to insure a low transfer frequency by transduction and transformation by reducing opportunities for illegitimate recombination" (59 FR 45548 (September 1, 1994)). Two of the commenters (#28 and 33) noted that sequences cannot be modified to decrease the likelihood of transformation, and that most transformation rates are above 10^{-8}. They expressed concern that no intergeneric *Bacillus* construct would meet the poorly mobilizable criterion, since genetic material from a *B. subtilis* chromosome will always be able to transform a recipient of the same taxon at frequencies well above 10^{-8}.

EPA response:

EPA agrees with those commenters who stated the 10^{-8} criterion at §725.421(c) is reasonable and prudent. It is a standard established by NIH in its Guidelines and an important feature of the OECD GILSP (Ref. 2). The 10^{-8} standard can be achieved readily and has been used by researchers for 20 years to ensure a measure of safety. EPA believes the 10^{-8} criterion should be applied to the introduced genetic material under § 725.421, because EPA is not limiting the source and function of the introduced genetic material (with the exception of § 725.421(d)). Therefore, EPA could not determine that certain categories of organisms (that otherwise qualified for the exemption) would present low risk without including the poorly mobilizable standard in the criteria at § 725.421. The poorly mobilizable criterion imposes a level of biological containment on the introduced genetic material and thus raises the Agency's confidence that a finding of "no unreasonable risk of injury to health or the environment" can be met for all organisms which qualify for the exemption under Subpart G. The poorly mobilizable criterion reduces the probability of gene transfer to microorganisms in the environment, should a limited number of the intergeneric microorganisms escape and survive in the environment.

EPA believes that manufacturers can readily determine whether the introduced genetic material will meet the 10^{-8} criterion. For many bacteria, a single mechanism of gene exchange, conjugation, will need to be considered and the introduced genetic material constructed to meet the 10^{-8} standard for that mechanism. Many of the bacteria used for fermentation applications reviewed under TSCA have had plasmid-borne intergeneric genetic material introduced into them, making conjunction the most likely mechanism by which the microorganism could transfer the intergeneric genetic material. The other two mechanisms, transformation and transduction, are less likely to widely disseminate introduced genetic material through the microbial community. For transduction and transformation, most introduced sequences will *a priori* meet the 10^{-8} criterion. However, for conjugation, determining the transfer rate for introduced genetic material when it is plasmid-borne may require closer consideration.

Transduction occurs primarily between members of the same strain or species of microorganism. Although some broader host range phages exist for some of the genera listed under § 725.420 such as *E. coli* (Ref. 16), generally bacteriophages have a narrow host range restricted to one

bacterial species or a small number of closely related bacteria (Ref. 17). The high level of specificity associated with transduction ensures a level of biological containment amongst taxa closely related to the intergeneric microorganism. Although transduction frequencies of up to 10^{-3} for *E. coli* in nonsterile soil have been noted, these frequencies were obtained under idealized conditions in which concentrations of phage, coliforms, and plasmids were optimized (Refs. 18, 19). A transduction frequency of 10^{-8} is probably more realistic for environmental conditions which are typically nonsterile and have varying concentrations of phage and recipient bacteria.

For transformation, realistic conditions for gene transfer in the environment would not likely include conditions that allow artificial transformation at high rates in the laboratory, such as high concentrations of calcium chloride and/or naturally competent recipients. Therefore, transformation frequencies in the environment are likely to be below 10^{-8}, although precautions must be taken to avoid the inclusion in the introduced genetic material of insertion sequences and/or transposons. Rates for natural transformation of *Bacillus subtilis* using chromosomal DNA have been reported in the range of 10^{-5} to 10^{-7} over a 1 to 15 day period (Ref. 20). However, these rates were obtained by optimizing conditions for transformation through the use of at least five different techniques: (1) the DNA for transformation was first adsorbed to the clay mineral montmorillonite prior to its introduction into the test system (this clay protects DNA from degradation); (2) the test system did not contain other bacterial species; (3) the *B. subtilis* recipients were made competent using special media prior to the test; (4) the recipients were introduced into the test system at a time when the cells attained maximum competency; and (5) optimal concentrations of recipients were present in the test system. Moreover, although genetic material that survives in the environment can move from one microorganism to another through transformation, a fairly high level of specificity for uptake, integration, and expression is associated with the recipient organism. Thus, some level of biological containment exists to limit genetic material being transferred to other microorganisms by transformation. To further ensure biological containment for these sequences, they must not contain known insertion sequences or transposons, because insertion sequences and transposons may allow easier integration of intergeneric genetic material into the recipient bacterium's genome. Where introduced genetic material does contain insertion sequences or transposons, submitters should consult EPA regarding eligibility under § 725.421.

For conjugation, one of three different levels of investigation may be needed to estimate a transfer frequency. For some bacteria, it will be a simple matter to ascertain whether the intergeneric introduced genetic material meets the poorly mobilizable criterion. For example, the transfer rate for the plasmid pBR322 in the absence of helper plasmids is below 10^{-8}. Therefore, in most cases using pBR322 as the vector no testing would be necessary to determine if introduced genetic material borne by pBR322 meets the criterion. In other instances, tests done under optimum conditions (for example, laboratory tests in the absence of indigenous microorganisms), may show that the transfer rate is below 10^{-8}. Finally, it may be necessary in some cases to test under realistic environmental conditions.

Some commenters were concerned about whether introduced sequences stably integrated into the

chromosome with no functional transposons meet the 10^{-8} criterion. Transfer frequencies for chromosomally integrated sequences are likely to be low, and can be reduced further by ensuring that the introduced genetic material does not contain insertion sequences or transposons. Genetic material stably integrated into the chromosome with no functional transposons is likely to meet the 10^{-8} criterion.

If companies encounter difficulties in reaching a conclusion on whether an intergeneric microorganism meets the criterion under § 725.421(c), they are encouraged to consult with the EPA.

d.Free of certain sequences

The requirements for the "free of certain sequences" criterion were set forth at proposed § 725.421(d) which indicated that the introduced genetic material must not contain any part of the

nucleotide sequences that encode certain listed toxins, which are polypeptides of relatively high potency. EPA discussed its rationale supporting the "free of certain sequences" criterion in the preamble to the proposed rule (59 FR 45547 (September 1, 1994)).

Comments:

EPA received one comment (#25) on this criterion. The commenter noted that the language in proposed § 725.421(d), if taken literally, would "preclude the use of DNA that codes for a pair of amino acids (or even a single one) if that sequence also occurs in any of these toxins". In order to clarify this point, the commenter suggested that the language be altered to state that the introduced genetic material must not contain a sequence "encoding any active moiety of a toxin" listed in § 725.421(d).

EPA response:

EPA did not intend for the restriction on toxin-encoding sequences to be interpreted to mean that the presence of a nucleotide found in a toxin gene sequence on the list at § 725.421(d) would preclude introduced genetic material containing that nucleotide from qualifying for the tiered exemption. EPA believes the likelihood of any significant risk resulting from incorporation of nonfunctional portions of a toxin gene into a recipient microorganism listed at § 725.420 is low. The commenter's suggested focus on "active moieties" is consistent with EPA's intention. However, an "active portion or moiety" of a toxin is terminology already in use in the literature, and that use is more narrowly defined in the literature than EPA's concerns. Therefore, EPA has modified the language of § 725.421(d) to include the term "functional portion" of a toxin-encoding sequence. To assist submitters in interpreting the term "functional portion" of the toxin-encoding sequences described at § 725.421(d), examples are provided below.

For toxins which affect a cell's cytoplasmic functions, nucleic acid sequences which form the

"functional portion" of a toxin are those which encode either functional A or B subcomponents of the toxin. Each member of this group of toxins consists of an A, or "active" portion which is responsible for the toxic action, and a B or "binding" portion which permits binding of the toxin to the target cell membrane. Some toxins have the A and B activities expressed by the same polypeptide, and others have separate polypeptide subunits for the two functions. Examples of these toxins, which act intracellularly, include those such as diphtheria toxin (included in the list of protein synthesis inhibitors at § 725.421(d)(1)), botulinum toxin (included in the list of neurotoxins at § 725.421(d)(2)), and cholera toxin (included in the list of toxins affecting membrane function at § 725.421(d)(4)).

For toxins which affect a cell's membrane, nucleic acid sequences which shall not be included in the introduced genetic material are those which encode the functional portion that allows target cell membrane disruption. The toxins listed under § 725.421(d)(5) function by affecting target cell membrane integrity. Membrane-disrupting toxins may act on the membrane directly; for example, lecithinase lyses cells indiscriminately by acting on the lecithin in mammalian membranes. A second group of cell membrane disrupting toxins act by forming protein pores in the membrane, such as streptolysin O, listed under § 725.421(d)(3), which binds to cholesterol in red blood cell membranes resulting in membrane channels. In contrast to those toxins which act on cytoplasmic functions, these cell surface-acting toxins are highly variable in structure and mode of action.

The same principle, however, applies to the membrane disrupting toxins as to the toxins which affect cytoplasmic functions: the introduced genetic material must not contain a nucleotide sequence that encodes a functional portion of any of these toxin sequences. In the case where a single polypeptide comprises the toxin such as with lecithinase, the functional toxin must not be present in the introduced genetic material. If a toxin subunit itself is comprised of different polypeptides, the introduced genetic material shall not encode a functional polypeptide from the toxin subunit. As an example, pertussis toxin which affects membrane function has a B subunit oligomer, which itself is comprised of four different polypeptides (referred to as the S2 - S5 polypeptides). The introduced genetic material must not encode functional portions of any one of these polypeptides.

The introductory text of § 725.421(d) has been modified to include a definition of the term "functional portion of a toxin-encoding sequence" and to emphasize that EPA is excluding specific toxin sequences and not source organisms. This explanation contains examples of sequences that directly or indirectly contribute to toxic effects in human cells; further discussion of these effects may be found in Ref. 21. The toxin list was developed based on the best literature available at the time. Any newly discovered toxins of high potency which are adequately described in the literature could be added to this list through notice and comment rulemaking. In order to further clarify the identity of the sequences of concern at § 725.421(d), the following text below has been substituted in the introductory portion of paragraph (d).

"(1) The introduced genetic material must not contain a functional portion of any of the toxin-encoding sequences described in this paragraph (d). (i) For the purposes of this section, a

functional portion of a toxin-encoding sequence means any sequence which codes for a polypeptide that has one of the following effects:

(A) It directly or indirectly contributes to toxic effects in humans. Directly contributes to toxic effects in humans means those sequences encoding polypeptides that have direct toxicity to target cells. An example of a sequence which directly contributes to toxic effects in humans is one which encodes the portion of diphtheria toxin, listed in paragraph (d)(2) of this section, capable of interacting with elongation factor 2, leading to inhibition of protein synthesis in target respiratory, heart, kidney, and nerve tissues. Indirectly contributes to toxic effects in humans means a sequence whose encoded polypeptide is not directly toxic to target cells, yet still adversely affects humans. An example of a sequence which indirectly contributes to toxic effects is the sequence which encodes the portion of the botulinum toxin, listed in paragraph (d)(3) of this section, capable of blocking the

release of acetylcholine from gangliosides. Botulinum toxin affects neuromuscular junctions by its blockage of acetylcholine release, leading to irreversible relaxation of muscles and respiratory arrest.

(B) It binds a toxin or toxin precursor to target human cells.

(C) It facilitates intracellular transport of a toxin in target human cells.

(ii) While these toxins are listed (with synonyms in parentheses) in paragraphs (d)(2) through (d)(7) of this section according to the source organism, it is use of the nucleotide sequences that encode the toxins that is being restricted and not the use of the source organisms. The source organisms are listed to provide specificity in identification of sequences whose use is restricted. Although similar or identical sequences may be isolated from organisms other than those listed below in paragraphs (d)(2) through (d)(7) of this section, these comparable toxin sequences, regardless of the organism from which they are derived, must not be included in the introduced genetic material."

4.Containment

The proposal included the following containment requirements for the Tier I exemption at § 725.422: (1) The structure is designed and operated to contain the microorganism, (2) limit entry only to those persons whose presence is critical to the reliability or safety of the activity, (3) provide written, published, and implemented procedures for the safety of personnel and control of hygiene, (4) provide and document effectiveness of inactivation procedures to reduce microbial concentrations by at least 6 logs in liquid and solid wastes, (5) provide and document effectiveness of features to reduce microbial concentration by at least 2 logs in aerosols and exhaust gases released from the structure, (6) include and document systems for controlling dissemination of the microorganisms through other routes, (7) have in place emergency clean-up procedures.

EPA received 10 comments (#8, 18, 23, 25, 28, 33, 34, 35. 39) on its containment criteria for the Tier I exemption. Two commenters (#18, 24) indicated that the standards for minimizing releases of microorganisms from facilities appeared to be reasonable. Six commenters (#23, 28, 33, 34, 35, 39) felt that some of the standards were too restrictive. Two commenters (#8, 25) felt that some of the standards were too lenient. The comments focussed on the limited entry requirement and the inactivation requirements.

a.Limited entry requirement

Comments:

Four commenters (#28, 33, 24, 25) indicated that the requirement to limit entry to the facility to critical personnel is unduly restrictive, given the low potential hazards posed by the microorganisms eligible for the Tier I exemption. The commenters felt that under that requirement, managers may be precluded from allowing administrative personnel, customers, and school and other educational tours into the facility. The commenters suggested substituting the following requirement: "(b) There is controlled access to the structure."

EPA response:

EPA recognizes that language at proposed § 725.422(b) may have been stricter than was necessary. Neither the NIH Guidelines (Ref. 11) nor the OECD GILSP criteria (Ref. 2) have specific limited entry requirements for large scale uses of comparable microorganisms. Additionally, EPA's review of PMNs received for intergeneric microorganisms indicated that restricting entry to critical personnel was not common industry practice (Ref. 22). EPA agrees with the commenters who stated that given the low risk posed by the microorganisms eligible for the exemption, managers should have the discretion to allow administrative personnel, customers, and school and other educational tours into the facility. However, EPA also expects that managers will maintain appropriate containment, thereby controlling access and avoiding inadvertent exposure. Modification of the language of this requirement does not alter EPA's original determination that microorganisms that are eligible for and used under the conditions of the Tier I exemption will not present an unreasonable risk of injury to human health and the environment. Therefore, EPA has revised § 725.422(b) to read "Control access to the structure."

b.Inactivation requirements

Comments:

Three commenters (#28, 33, 35) indicated that with the limitations placed on the recipient microorganism and the introduced genetic material, quantitation of inactivation procedures was not necessary. The commenters stated that it would be necessary to modify existing equipment to sample off-gas as required and that an additional sample port would increase the potential for contamination and worker exposure. The commenters suggested that instead of numerical

requirements, language be substituted that more generally required reduction of microorganisms in liquid and solid wastes and aerosols and exhaust gases.

Two commenters (#8, 25) felt that the numerical requirements for the inactivation procedures are too lenient. These commenters suggested that gases be vented through a HEPA filter or incinerated. They also recommended that the containment criteria be coordinated with the containment levels set out in the NIH Guidelines.

EPA response:

After considering comments regarding its inactivation requirements at proposed § 725.422(d) and (e), EPA reviewed information submitted on physical containment and control technologies in PMNs it has received for intergeneric microorganisms between 1986 and 1995 (Ref. 22). On the basis of that review, EPA has made the following determinations. EPA has decided to retain § 725.422(d) which requires the use of inactivation procedures that reduce microbial concentrations by at least 6 logs in liquid and solid wastes. However, EPA has determined that it is appropriate to revise § 725.422(e) to read "Use features to minimize viable microbial populations in aerosols and exhaust gases released from the structure, and document use of such features." The reasons for the decisions on these two requirements are discussed separately in more detail below.

Control of liquid and solid wastes. As indicated in the preamble to the proposed rule (59 FR 45548-49 (September 1, 1994)), EPA believed that it was appropriate to prescribe standards for minimizing the number of microorganisms emitted through the disposal of wastes, because a wide range of behaviors could be displayed by microorganisms eligible for the exemption and because EPA would not be reviewing MCANs on microorganisms eligible for the Tier I exemption. EPA believes that the requirement for a 6-log reduction in the number of microorganisms is reasonable for inactivation of liquid and solid wastes and well within current industry practices. The 6-log reduction criterion represents a level of inactivation which can be validated. Reductions greater than 6-logs become increasingly more technically difficult to demonstrate. As discussed in the preamble to the proposed rule (59 FR 45548-49 (September 1, 1994)), the number of viable microorganisms remaining in the liquid and solid wastes after inactivation is expected to be much lower than that required by the 6-log reduction. An examination of PMNs for intergeneric microorganisms (Ref. 22) revealed that this criterion can be readily achieved by manufacturers. The review of these PMNs also indicated that in the several cases where monitoring was conducted there were no detectable viable microorganisms in liquid and solid wastes after inactivation (Ref. 22).

For proprietary reasons, manufacturers typically ensure complete inactivation of their liquid and solid wastes. Even if some small number of viable cells below the detection limit remained subsequent to inactivation, their survival outside the fermentation facility is likely to be limited since microorganisms used for extended periods under cultivation typically become adapted to the optimal nutritional and physical conditions in the fermentor. Their ability to survive in the

environment is compromised. In addition, further treatment of the wastes such as in publicly owned treatment works (POTWs) greatly lessen the opportunity for survival. Therefore, EPA believes that the 6-log reduction in viable microbial numbers in the liquid and solid wastes is a reasonable and demonstrable performance criterion ensuring an appropriate level of containment for the low risk microorganisms which would be eligible for the tiered exemption.

Validation of the 6-log reduction in microbial numbers for the liquid and solid wastes need not be conducted for each fermentation batch. Once an inactivation procedure has been documented as being validated for meeting the 6-log reduction, that same inactivation procedure can be followed to meet the required performance criteria.

EPA did not mean to imply that the required 6-log reduction referred to a 6-log overkill capacity of an inactivation procedure. The 6-log reduction in the number of microorganisms in the liquid and solid wastes meant that the inactivation procedure used must reduce viable microbial numbers by 99.9999%. This is equivalent to a 10^{-6} probability of a microorganism surviving an inactivation treatment. As indicated in the preamble, EPA expects the concentration of microorganisms in fermentation vessels to range up to 10^{10} to 10^{11} colony forming units (CFU)/ml. A simple calculation assuming no dilution of the fermentor broth and a minimum inactivation efficiency of 99.9999 percent (or 6-log reduction) results in an estimate of the concentration of viable organisms released from the facility of at most approximately 10^{-5} bacteria per milliliter. However, this number is likely to be lower, since the required reduction is the minimum validated inactivation and the actual kill achieved, shown by developing an inactivation curve which is a standard industry practice, is likely to be greater. Additionally, as discussed above, the survival in the environment of any microorganisms remaining after inactivation is likely to be compromised.

Control of aerosols and exhaust gases. As indicated in the preamble to the proposed rule (59 FR 45548-49 (September 1, 1994)), EPA also believed that it was appropriate to require manufacturers to minimize the number of microorganisms emitted through the venting of gases because a wide range of behaviors could be displayed by microorganisms eligible for the exemption and because EPA would not be reviewing MCANs for microorganisms eligible for the Tier I exemption. In the proposal EPA indicated that a 2-log reduction in viable microorganisms per cubic foot of air between the headspace and the actual vent port was the appropriate standard. EPA chose this number based on an estimate of the numbers of microorganisms likely to be in the exhaust from an uncontrolled fermentor and common industry practice, and because it represented a somewhat less restrictive number than the reduction obtained with HEPA filter filtration (the reduction level required for the NIH Guidelines BL1-LS level (Ref. 11)).

However, EPA received several comments pointing out the technical problems associated with the proposed 2-log reduction performance criterion. EPA agrees with the commenters that companies should not have to modify/retrofit their existing equipment nor jeopardize the sterility of their fermentations in order to validate that the number of microorganisms being released in the

exhaust has been reduced by at least 2 logs relative to the microbial numbers in the fermentor gases in the headspace. EPA did not intend that retrofitting or any other overly burdensome engineering modifications would be necessary for those who wished to utilize the Tier I exemption. Rather, EPA had intended to develop requirements that would impose performance standards for equipment already commonly used. In light of comments received, EPA has sought to modify its requirement to achieve its goal of having manufacturers demonstrate that the equipment or features normally employed in fermentation systems are effective in reducing numbers of viable microorganisms being vented in exhaust gases.

As stated in the preamble and noted by two commenters (#28, 33), industrial fermentations are not routinely run in an uncontrolled fashion, and thus the number of microorganisms potentially released into the gas phase and unrecovered is controlled. Additionally, an examination of PMNs for intergeneric microorganisms (Ref. 22) showed that all of the fermentations were operated with features which minimize the number of microorganisms released in the off-gases.

For fermentations to operate optimally, vapor recovery systems are used to maintain the correct growth conditions for the microorganisms, e.g., correct molality in the fermentation broth must be maintained. Vapor recovery systems, by their nature, help to minimize the number of microorganisms exhausted from the facilities. EPA believes that it should allow some flexibility in the type of features manufacturers employ to minimize microbial releases as aerosols. A variety of fermentor equipment or features are commonly used by the industry such as demisters, wet scrubbers, cyclone separators, coalescing filters, and HEPA filters. As stated in the preamble (59 FR 45549 (September 1, 1994)), even if microorganisms are exhausted from the fermentor, their survival is probably limited due to the stress conditions of aerosolization, including shear forces, desiccation, and UV light exposure.

Given the comments received on the feasibility of this requirement and the variety of methods used by PMN submitters to reduce microbial numbers in aerosols, EPA believes that a specific numerical performance standard is less appropriate for inactivation of aerosols than it is for inactivation of liquid and solid wastes. EPA agrees with commenters (#28, 33) who asserted that the majority of microorganisms potentially released from the fermentation facility would be found in the liquid and solid wastes. EPA has prescribed a specific viable microorganism reduction standard for these materials. Therefore, EPA believes that if the new microorganism meets all of the other requirements of the Tier I exemption, it is sufficient to require use of effective methods for minimizing release of microbial concentrations in aerosols and exhaust gases without prescribing a specific numerical reduction in numbers. If manufacturers are conducting their quality assurance/quality control (QA/QC) monitoring to ensure proper performance of their fermentation equipment, EPA believes that the facilities would be meeting the requirement of § 725.422(e). EPA has revised § 725.422(e) to read "Use features known to be effective in minimizing viable microbial populations in aerosols and exhaust gases released from the structure, and document use of such features." Based on the above points as well as the results of EPA's review of past microorganism PMNs, EPA believes that this requirement will ensure that the number of microorganisms released in fermentor off-gases will be negligible and allow EPA to

make the "no unreasonable risk" finding of § 5(h)(4). As discussed in the preamble to the proposed rule (59 FR 45542 (September 1, 1994)), in order to promulgate a TSCA § 5(h)(4) exemption, EPA must determine that activities involving new microorganisms eligible for the exemption will not present an unreasonable risk of injury to human health and the environment.

EPA does not agree with commenters who stated that a 2-log reduction for aerosols is too lenient. As discussed in the preamble (59 FR 45549 (September 1, 1994)), even if small numbers of microorganisms are released in fermentor exhaust gases, aerosolization is a stressful condition decreasing the survival of most microorganisms. Aerosolized bacterial cells are weakened by shear forces, and are subject to desiccation and exposure to UV light. Therefore, survival of aerosolized microorganisms is expected to be limited. Since the organisms which are eligible as recipient microorganisms for the Tier I exemption are low risk, EPA does not believe it is necessary to impose more stringent conditions than a requirement that manufacturers use validated methods to minimize the numbers of microorganisms in fermentor off-gases.

Several commenters (#8, 18, 25, 39) suggested that EPA coordinate its containment criteria with those specified in the NIH Guidelines. EPA considered use of the NIH Guidelines when it was developing the tiered exemption but found such an approach to be problematic. In particular, the NIH Guidelines may change through a process independent of EPA such that the Guidelines would no longer provide the appropriate criteria to support a TSCA § 5(h)(4) exemption. EPA has developed an approach at § 725.422 based, in large part, on standards set forth in the NIH Guidelines and the OECD GILSP (Ref. 2) that allow EPA to make the finding that is required under § 5(h)(4). In considering the specific containment requirements of the current NIH Guidelines (Ref. 11), EPA could not find one level from Appendix K that EPA believed would be appropriate for the Tier I exemption. The NIH Good Large Scale Practice (GLSP) criteria that would be applicable to some, but not all, of the microorganisms listed at § 725.420, do not require minimization of the numbers of microorganisms released in off-gasses. However, EPA finds a requirement to minimize the number of organisms in off-gases to be appropriate. On the other hand, Biosafety Level 1-Large Scale (BL1-LS) criteria, which would be too stringent for some of the microorganisms listed at § 725.420, require the use of HEPA filters or their equivalent, which generally provide at least a 3-log reduction (Ref. 25). EPA finds the BL1-LS requirement, which is more rigorous than EPA's original 2-log reduction requirement, to be more restrictive than EPA believes is necessary for the Tier I exemption.

In reconsidering its proposed requirement, EPA believes that the costs of retrofitting existing equipment as well as the increase in potential contamination and worker exposure that would accompany sample collection necessary to validate the 2-log reduction requirement are not justified for the low risk microorganisms eligible for the Tier I exemption. EPA has attempted to make its approach compatible with good practice in industry. Most of the requirements of §725.422 are analogous to NIH Guidelines requirements. In particular, companies who are in full compliance with the NIH BL1-LS requirements would also be in compliance with §725.422(e), although the use of HEPA filters or their equivalent is a more stringent requirement than §725.422(e).

IV. REPORTING R&D ACTIVITIES FOR TSCA MICROORGANISMS

As discussed earlier in this document and in the proposed rule, TSCA § 5 generally requires notification of EPA at least 90 days prior to the manufacture and importation of new chemical substances and 90 days prior to the manufacture, importation, and processing of designated chemical substances for significant new uses. TSCA § 5(i) makes clear that only manufacturing, importing, and processing "for commercial purposes" are subject to § 5 notification. TSCA § 5(h)(3) exempts entirely from notification under § 5 the manufacturing, importing, and processing of chemical substances "only in small quantities (as defined by the Administrator)" for R&D, subject only to the manufacturer, importer, or processor notifying (as prescribed by EPA) the persons involved in the R&D activity of any risks to health associated with the substance.

As discussed in more detail below, for traditional chemical substances, EPA has defined "small quantities" for R&D to be those quantities "not greater than reasonably necessary" for the R&D purposes. However, EPA is adopting a different definition of "small quantities" for R&D for microorganisms, because living microorganisms may reproduce and increase their own numbers or amount. The definition adopted in this final rule limits the § 5(h)(3) exemption from § 5 MCAN requirements to R&D activities that are adequately contained as set forth in § 725.234.

This narrower definition of "small quantities" means that R&D activities conducted outside the prescribed containment (including field tests) do not qualify for the § 5(h)(3) exemption and are subject to the MCAN requirement. However, EPA has created, under authority of TSCA § 5(h)(4), other exemptions that will reduce the reporting burden for persons conducting certain R&D activities that do not qualify for the complete exemption in § 5(h)(3). These are discussed below.

Researchers, including those in academic institutions, may be subject to TSCA § 5 jurisdiction because, by creating or reproducing microorganisms in their R&D activities, they are "manufacturing" or "processing" such microorganisms. Since many such R&D activities involving microorganisms will not qualify for the § 5(h)(3) exemption from MCAN reporting, it is important for researchers, including those in academic institutions, to determine whether their activities fit within the definition of "commercial purposes" and, thus, are subject to TSCA § 5 and the MCAN requirements. Because of the nature of microorganism R&D and the definition of "commercial purposes" discussed below, it is likely that many researchers, including some in academic institutions, will be subject to TSCA § 5 jurisdiction for the first time and will want to utilize the TERA and other exemption provisions to reduce the reporting burdens involved in their R&D activities.

Each of the exemptions for R&D activities applies to specific types of activities. At the beginning of R&D, while the research is taking place in a laboratory subject to appropriate containment, the R&D activity may be fully exempt under the § 5(h)(3) exemption if the researcher complies with the conditions set out in the rule. Once the researcher decides to conduct research outside the contained setting, such as field tests, the researcher will need to utilize a different exemption, such

as the TERA.

A. TSCA JURISDICTION

The first step in determining potential TSCA § 5 obligations for R&D activities is to ascertain whether the use or potential use of the microorganism is specifically excluded from TSCA § 5. Uses that are not specifically excluded are subject to TSCA. This consideration is discussed in greater detail in this document in Unit II.

Comments:

EPA received four comments (#11, 24, 31, 34) regarding TSCA § 5 coverage of R&D activities with microorganisms. One commenter (#34) expressed concern that:

Any discovery and range-finding development work would be reportable. Under the strictest interpretations, ETA would expect industry and academia to submit a large number of unnecessary reportings and maintain documentation for work which would not ultimately culminate in a product to be introduced into commerce.

Another commenter (#24) stated that "some clarification of intent is required where EPA asserts that all R&D is eligible for TERA reporting. Much R&D activity falls outside the jurisdiction of TSCA and therefore would not be subject to TERA." One commenter (#11) requested clarification of EPA's statement in the proposal that "EPA would consider that R&D activities involving new microorganisms where researchers are unsure of the final use would be subject to TSCA § 5." This commenter went on to state that "based on this statement, some biotechnology companies whose purpose is the development of new drugs have expressed concern that the proposed rule may apply to the early stages of their research." This commenter (#11) requested that EPA confirm that entities performing research with new microorganisms for the purposes of developing new drugs would not be subject to TSCA. Another commenter (#31) requested that EPA confirm that the proposed rule does not cover "Intergeneric microorganisms that are cloned for use in research leading to possible new plant varieties for food and other uses."

EPA response:

With regard to the clarification requested by one commenter (#24), EPA's statement that all R&D activities are eligible for reporting using the TERA process refers solely to R&D which is subject to TSCA § 5 requirements. EPA anticipates that much R&D activity with microorganisms will not be subject to TSCA.

With regard to the commenter's (#11) requested clarification on the status of R&D activities involving new microorganisms "where researchers are unsure of the final use," EPA explained in the proposed rule that, unless specifically excluded by TSCA, microorganisms will generally be subject to TSCA. As explained in more detail in Unit II, TSCA specifically excludes from its

jurisdiction, various chemical substances that are employed for particular products or "uses". Microorganisms, or chemical substances, that are produced for multiple or undifferentiated uses that are not specifically excluded, however, are generally subject to TSCA. This is consistent with EPA's treatment of traditional chemicals; in the original Inventory Reporting Rules EPA stated:

[i]f a substance has multiple uses only some of which are regulated under FIFRA or FFDCA, the manufacture, processing, distribution, and use of the substance for the

remaining uses would come within the jurisdiction of TSCA. In cases where a substance is manufactured, processed, or distributed for undifferentiated uses, the substance will be presumed to be subject to TSCA for the purposes of these regulations.

42 FR 64585 (December 23, 1977).

With regard to biotechnology companies engaged in development of drugs, as explained in more detail in Unit II, microorganisms used in the production of foods, drugs, cosmetics and medical devices are similarly excluded from TSCA. Research conducted with the intention of developing a product that would be solely employed as one of those specifically excluded uses, would not be subject to TSCA. However, persons who perform research with the intention of developing a microorganism that may have multiple uses, some of which would be subject to FIFRA or TSCA, or who are unsure of the ultimate use, will need to consider whether they are subject to TSCA.

With regard to comments (#31) about the status under TSCA of intergeneric microorganisms used in research leading to possible new plant varieties for food and other similar research, EPA responds that if the plants are being developed solely for a use specifically excluded by TSCA, such as food, intergeneric microorganisms used in the production of those plants would not be subject to TSCA. However, if intergeneric microorganisms are used in the development of plants whose use is uncertain, those intergeneric microorganisms could be subject to TSCA.

However, EPA did not intend to imply that researchers using microorganisms would automatically be subject to § 5 requirements without consideration of whether the research was conducted for a commercial purpose. As discussed in Unit IV.B. below, if the research is at such an early stage that any product development is highly speculative, the research may not be "for commercial purposes", and thus may not be subject to TSCA § 5. The commenters apparently misunderstood EPA's proposed preamble discussion, which was intended only to explain the analytical steps to follow in determining whether researchers would be required to file a TERA notice.

In addition, if the research activity is deemed to be commercial, the research activity may be eligible for exemption under TSCA § 5(h)(3) (see Units IV.C. and E.), if the microorganisms are used exclusively for R&D and meet the other requirements of the exemption.

B. RESEARCH FOR COMMERCIAL PURPOSES

The most substantial decision made in the final rule was selection of the definition of commercial purposes for R&D activities. TSCA § 5(i) limits all § 5 screening to activities for commercial purposes. Research on traditional chemicals is not generally affected by the commercial purposes limitation, because EPA's current regulatory definition of small quantities for R&D using traditional chemicals ("any amounts reasonably necessary for research") at § 720.3 effectively exempts most research with these chemicals from § 5 review. However, because of the ability of microorganisms to reproduce, disseminate and spread, EPA believed that it was necessary to review these products at an earlier stage and therefore proposed an interpretation to address testing with microorganisms. Consequently, EPA developed a different small quantities definition for microorganisms and is imposing reporting and record keeping requirements on certain R&D activities. Researchers utilizing microorganisms, therefore, will need to consider whether their R&D activities would be considered commercial, and therefore subject to TSCA § 5 requirements.

During development of regulations on biotechnology over the past several years, EPA has received numerous public comments that differ substantially on how the Agency should apply the commercial purposes definition to research. Of particular concern has been the propriety of an EPA oversight system based on the status of an activity as commercial or noncommercial rather than on potential risk. Because of the past difference in public opinion, EPA proposed three potential approaches to defining what constitutes commercial activities: (1) using indicia to determine commercial purposes; (2) presuming all environmental testing is commercial; and (3) presuming that all environmental research is commercial but offering an opportunity for researchers to rebut the presumption. Rather than indicating a preference, EPA discussed in the preamble the advantages and disadvantages of each approach and asked for public comment on which approach would be appropriate.

The first approach is based on the usual way of interpreting a statutory term of art like "commercial purposes"; i.e., looking for indicia of commercial intent. This is what EPA does in its TSCA § 5 program for traditional chemicals. EPA recognized in the proposal discussion of this approach the often complex relationships between academia and industry in biotechnology, and the difficulties raised by such relationships in determining what constitutes commercial R&D activities at noncommercial institutions.

Under the second approach, all intentional testing outside of contained structures would be commercial. This approach avoided the most significant problem identified by comments over the years - that regulatory oversight should be based on risk and that there is no difference in the potential for risk between research conducted by industry and research by noncommercial entities. EPA also included a third approach which allowed researchers to rebut the presumption of the second approach that their testing is commercial.

Comments:

EPA received 13 comments (#7, 8, 12, 18, 22, 23, 28, 29, 33, 34, 36, 37, 39) on its three alternative approaches to interpreting "commercial purposes" for R&D activities. Five commenters (#7, 8, 18, 22, 36) urged EPA to develop "clear and direct indicia" (#7), and strongly opposed the option of defining all environmental testing as commercial. One commenter (#7) took issue with the idea of potentially considering all research "commercial when conducted at an institution receiving any commercial funding, regardless of whether those funds are channeled to the project in question." Another commenter (#8) while encouraging EPA to clearly define commercial R&D, also indicated that "if criteria or clear indicia cannot be agreed upon, it may be necessary to require that all intentional testing outside of contained structures be considered commercial." One commenter (#29) expressed concern about potential "confusion among investigators at academic institutions who have research partnerships with biotechnology firms." This commenter sought clarification of the reporting status when a business provides research material but does not fund a specific field test.

Six commenters (#12, 24, 28, 33, 37, 39) urged that EPA not distinguish between commercial and non-commercial research for microorganisms. One of these commenters (#12) specifically supported the approach that defined all environmental research as commercial. Another commenter (#37) indicated that "it is questionable to consider all field tests to be commercial in intent in order to bring them under the regulatory purview of TSCA" but at the same time agreed "that there is no substantial difference in the risks posed by testing by academic/non-profit organizations and commercial institutions." The other four commenters were primarily concerned that "academic, government and non-profit organizations should be viewed the same as commercial enterprises with regard to the implementation of these policies" (#24). Two commenters (#28, 33) suggested that EPA resolve the dilemma posed by the contradictory directives imposed by TSCA (i.e., protect against risk and exempt non-commercial research) at the R&D stage by entering into an MOU with NIH in which NIH formally defers to EPA for environmental testing that would normally be covered by TSCA.

EPA response:

Comments received on the proposed rule produced no prevailing opinion on how EPA should define "commercial purposes" for R&D. In considering this issue, EPA turned to its experience over the past several years responding to researchers who inquired about the status of their field tests under TSCA. EPA based its responses to those inquiries, in part, on its approach to traditional chemicals under TSCA. Under the TSCA § 5 program for traditional chemicals, EPA determines whether an activity is for a commercial purpose based on whether the purpose of the activity is to have an immediate or eventual commercial advantage. EPA found that determining the commercial status of research microorganisms based on indicia similar to those used for traditional chemicals functioned adequately. Therefore, EPA has decided that for this final rule when determining whether their R&D activities with microorganisms would be "for commercial purposes," researchers will need to consider the indicia listed in § 725.205(b). The indicia approach applies to R&D in laboratories and other contained structures as well as to intentional testing in the environment and is discussed in more detail below.

Researchers who are attempting to determine whether their research would be for "commercial purposes" should consult § 725.205(b). Under § 725.205(b)(1) researchers would first consider whether any of the funding for the proposed research comes directly from a commercial source. Any direct industry involvement in or direct funding of an activity at a noncommercial institution is for commercial purposes. This would include the use of company funds to develop the microorganisms or the use of a company-provided microorganism in the research. If any portion of the research is funded directly by a commercial source, then the research is "for commercial purposes." Thus, if any part of the research is funded by contract, joint venture, or other financial arrangement, with the purpose of eventually producing a commercial product, the research is subject to the requirements of § 5. For example, laboratory work or field tests conducted under a research contract between a company and a university or a researcher where patent rights or trade secrets are held by the company, would be considered commercial R&D.

If researchers do not fall under § 725.205(b)(1), they should next consider potential indirect indicators of commercial intent as reflected in § 725.205(b)(2). They would need to consider, for example, whether the research is directed towards developing a commercially viable improvement of a product already on the market, or whether they are seeking commercial funding or a patent.

If researchers do not fall within the scope of § 725.205(b)(1) or (b)(2), their research may be considered noncommercial. For example, an outright gift from a company to a university or a researcher without the company directing or otherwise controlling the research for which the funds are to be used or the use to be made of the results of the research conducted, would not be considered direct funding under § 725.205(b)(1). As such, the research conducted using such a gift would be considered noncommercial R&D, assuming the researcher also does not believe the microorganism has the potential to be developed as a commercial product in the future, or otherwise intends to obtain an immediate or eventual commercial advantage as described under § 725.205(b)(2). Therefore, if a researcher is planning to conduct laboratory work or field tests or other environmental testing using funds which were part of an outright gift from a company to the university with no strings attached, that research would be considered noncommercial R&D.

If none of the funding or support for the laboratory work or field test or other environmental testing, including development of the microorganism, comes from a commercial source, then the researcher must consider whether he or she intends to pursue the development of the new microorganism as a commercial product in the future, should testing show potential commercial viability. The researcher is responsible for judging when commercial intent exists for his or her particular research project. EPA recognizes that in the initial stage of research projects, researchers may not envision an eventual commercial purpose for their microorganisms. However, if, during the course of their investigations, researchers determine that their microorganism has a potential commercial use which they intend to pursue, they then become subject to the requirements of TSCA § 5 and this rule, and their further research activities must be in compliance with this rule. EPA has provided examples of research that has an immediate or eventual commercial advantage in the final rule regulatory text at § 725.205(b)(2)(i) through (iv). An example of "other evidence" of a commercial application cited under § 725.205(b)(2)(iv)

would be if the researcher has engaged in serious discussion with a company concerning

marketing or commercializing the microorganism if initial research is successful. If researchers have difficulty deciding whether their research is for commercial purposes, they are encouraged to consult EPA.

The above approach represents a modified version of the indicia of commercial purposes approach discussed in the preamble to the proposed rule. EPA has adopted this modified version for the following reasons. All research conducted directly by a commercial entity is clearly for commercial purposes, as the court decided in The Dow Chemical Company v. EPA, 605 F.2d 673 (3d Cir. 1979). Consequently, if a business directly funds a research activity for potential product development, the activity is for commercial purposes, even if the research activity is conducted at an academic institution. EPA has chosen to focus on the source of funding for the specific laboratory work or field test or other environmental testing as the appropriate indicator of commercial intent, because EPA recognizes that it can be difficult to trace sources of funding at the institutional level and agrees with the commenter who stated that "there is no logical basis for the assertion that commercial support of one narrowly defined project changes the fundamental academic nature of every other activity conducted elsewhere in the institution."

The interpretation of commercial purposes EPA is choosing to implement is consistent with interpretations in the current regulations for traditional chemicals. That is, a commercial activity is one undertaken with the purpose of obtaining an immediate or eventual commercial advantage. This is the definition in § 720.3(r), which defines "manufacture or import for commercial purposes," and § 721.3, which defines "process for commercial purposes." Consequently, EPA has adopted the idea in § 725.3, which defines for microorganisms "manufacture, import, or process for commercial purposes." Similarly, § 720.30(i) provides that "non-commercial research and development" consists of activities conducted by academic, government, or independent not-for-profit organizations "unless the activity is for eventual commercial purposes." EPA has developed a comparable exclusion for non-commercial R&D uses of microorganisms by including a definition of "commercial purposes for research and development activities" at § 725.205(b). As noted above, this commercial indicia approach applies to R&D in laboratories and other contained structures, as well as to intentional testing in the environment.

EPA's experience over the past several years responding to researchers inquiring about the status of their environmental research under TSCA indicates the following points. All of the researchers identified the sources of their funding for the particular experiments. Generally they were able to readily indicate whether they believed there was a future commercial application for the microorganism. In most cases where a company was directly funding field tests to be conducted at university sites, the company contacted EPA directly and took responsibility for preparation of the PMN. In one case, researchers were being funded by Federal agencies but were using company-owned microorganisms subject to a TSCA § 5(e) consent order. The company asked EPA to modify the consent order to allow the company to give the microorganisms to the

researchers for use in their field tests. Although the company made the original request, the researchers submitted information about their field tests to EPA. Therefore, researchers should contact EPA if they are planning field tests involving intergeneric microorganisms supplied by a company. In most cases, the researcher would be expected to submit the TERA.

In several cases where researchers contacted EPA regarding the status of their field tests, EPA found that field tests using intergeneric microorganisms were not subject to TSCA, because the field tests were being funded by other Federal agencies and the researchers did not foresee future commercial uses for their microorganisms. Finding that these field tests did not constitute commercial R&D under TSCA, EPA directed the researchers to the Federal agencies that were the primary funding sources for the field tests and suggested that researchers should, at a minimum, obtain reviews from these agencies under relevant authorities, including the National Environmental Policy Act (NEPA).

Although EPA has chosen in this final rule to follow an approach for "commercial purposes" similar to its approach for traditional chemicals, EPA recognizes that there are no differences in risk depending on funding source. EPA takes seriously its responsibilities to address risk and intends to pursue approaches laid out in the Coordinated Framework for Regulation of Biotechnology (51 FR 23302, June 26, 1986) to ensure an adequate network of oversight of R&D activities. To this end, EPA will work closely with other agencies, particularly NIH.

C.MICROORGANISMS ELIGIBLE FOR THE R&D SMALL QUANTITIES EXEMPTION

TSCA § 5(h)(3) exempts from § 5 screening chemical substances manufactured or processed in small quantities solely for R&D and directs EPA to define small quantities by rule. EPA's regulations for traditional chemicals at § 720.3(cc) define "small quantities solely for R&D" as those quantities that are "not greater than reasonably necessary for ...[R&D] purposes." This definition of small quantities for R&D has been appropriate for traditional chemical substances, because these chemicals do not have the ability to increase their own volume or amount. However, living microorganisms may reproduce and increase beyond the number initially introduced, may establish in the environment, and may spread beyond the test site. Once they are released into the environment or are no longer contained, there is no longer an assurance they will remain small quantities.

Therefore, EPA's definition at § 725.3 of "small quantities" for microorganisms is restricted to microorganisms that meet the requirements of § 725.234, which are designed to reduce the probability of establishment by reducing the number and frequency of viable microorganisms emitted from a facility. The small quantities exemption for microorganisms is also referred to as the "contained structures" exemption, because § 725.234(c) limits the exemption to R&D activities in contained structures.

EPA received one comment (#33) regarding EPA's authority under TSCA § 5(h)(3) and five comments (#21, 31, 33, 38, 39) regarding the status under TSCA of certain types of microorganisms used in research. Generally these concerns related to use of research microorganisms in commerce and use of genetic libraries.

1. EPA's authority under TSCA §5(h)(3)

Comments:

One commenter (#33) argued that "Section 5(h)(3) of TSCA permits manufacturers to manufacture or process small quantities of material for the purposes of R&D" and argued that "there is substantial burden on the Agency to justify any wholesale removal of the R&D exemption provided by Congress."

EPA response:

EPA disagrees with the commenter (#33) who stated that EPA had not justified the "wholesale removal of the R&D exemption provided by Congress." TSCA § 5(h)(3) does not provide a complete exemption for all R&D, nor has EPA removed the statutory exemption wholesale. Rather, TSCA § 5(h)(3) exempts from § 5 reporting, chemical substances manufactured or processed in small quantities for R&D and specifically directs EPA to define small quantities by rule. EPA has determined that the definition of "small quantities" applied at § 720.3 to traditional chemical substances cannot be applied to all R&D activities involving microorganisms for the reasons discussed in the preamble to the proposed rule (59 FR 45539-40 (September 1, 1994)). While discrete amounts of traditional chemicals used for R&D will be diluted in the environment, living microorganisms intentionally released to the environment have the potential to reproduce and increase beyond the amount originally released. Therefore, EPA has utilized its authority under TSCA § 5(h)(3) to define "small quantities" for living microorganisms differently than for traditional chemical substances. By imposing regulatory conditions to reduce the probability of establishment, EPA increases the probability that a living microorganism will remain a small quantity used solely for the purpose of R&D.

2. Use of research microorganisms in commerce

Comments:

Four commenters (#21, 31, 33, 39) specifically asked whether the traditional chemicals approach which applied the § 5(h)(3) exemption to research chemicals sold in commerce would apply to microorganisms subject to TSCA. One commenter (#31) stated the following:

"EPA must therefore confirm that Inventory and PMN-exempt research and development microorganisms include microorganisms which are manufactured/imported commercially and sold for use as:

1.research products for experimentation on other substances;

2.intermediates for the manufacture of research chemicals;

3.analytical standards,

4.quality control agents;

5.reagents used for chemical identification including commercial "test kits" which are used outside of a laboratory and in the field to identify or quantify the presence of other "traditional chemicals" or microorganisms in products, other substances, or in environmental media. Such "test kits" meet the TSCA 5(h)(3) definition of exempted research and development which EPA has consistently applied to the sale of R&D substances.

6.microorganisms which are manufactured under prudent laboratory practices inside a laboratory in small quantities at laboratory scale for commercial sale for research purposes."

EPA response:

Although EPA redefined "small quantities" to address the specific properties of microorganisms, EPA has not redefined or reinterpreted the phrase "solely for research and development" and intended that it would be applied to activities for microorganisms in the same way that it has been applied to traditional chemicals. This is because there is no significant difference in the type of research and development activities described by this phrase for microorganisms as compared to traditional chemicals. Consequently, any "small quantity of microorganisms," i.e., microorganisms which meet the requirements of § 725.234, may be used for any activity for which a traditional chemical eligible for the exemptions at §§ 720.36 and 720.78 could be used.

For purposes of clarification, EPA has modified requirements originally included in proposed § 725.235. Most of the proposed language was adapted, with little revision, from the small quantities exemption for traditional chemicals at § 720.36. Upon further reflection, EPA has determined that some of that language is not appropriate for microorganisms. Therefore, EPA has deleted proposed § 725.235(a)(2), which provided an exemption from the small quantities notification requirements for R&D in a laboratory, and proposed § 725.235(e), which related to impurities and articles. Additionally, the requirements at proposed § 725.235(c), (d), and (f) have been moved to § 725.205(d), (e), and (f), respectively, as these requirements apply to all R&D activities under Subpart E. EPA has further revised § 725.205(f) to specifically exclude microbial pesticides by referring to the microbial pesticide notification requirements that were promulgated in September 1994 (59 FR 45612).

Because EPA modified the definition of "small quantities" for microorganisms, there will be some differences between the way EPA's regulations treat R&D involving traditional chemicals and R&D involving microorganisms. For this reason, and because EPA is uncertain what the

commenter (#31) meant by the phrase "Inventory and PMN-exempt research and development microorganisms," EPA cannot confirm that all six of the categories listed by that commenter will meet the exemption requirements listed in §§ 725.234 and 725.235.

In general, an activity will be considered "research and development" if it consists of scientific experimentation or analysis for purposes of developing a product (the discussion of commercial purposes under Unit IV.B in this document is also relevant here). EPA intends this phrase to be interpreted broadly, because EPA recognizes that contained microorganism R&D may consist of several types of activities, including the bench-level or laboratory synthesis stage, pilot plant stage, and the performance testing stage. R&D activities include tests of the genotypic, phenotypic, production and performance characteristics of the new microorganism. For example, tests of genotypic and phenotypic properties usually occur at the laboratory stage. Tests of production characteristics could include tests of manufacturing equipment and production processes. Tests of performance characteristics for R&D purposes could include carefully monitored tests using limited quantities of the microorganisms by the manufacturer or potential customer. These tests are distinguished from test marketing activities which usually involve sale or distribution to potential customers to determine competitive value of the substance. Any of these activities which are not test marketing activities and occur in facilities meeting the small quantities/contained structures exemption are eligible for the exemption.

Sale of a microorganism for use in R&D does not necessarily make the microorganism ineligible for the R&D small quantities exemption. To be eligible for the R&D small quantities exemption, microorganisms must be produced and used exclusively for R&D. Microorganisms which are sold exclusively for the purpose of research and development activities are eligible for the R&D small quantities exemption if they meet the requirements of § 725.234. Manufacturers, importers, and processors may derive compensation from the sale of microorganisms on which R&D will be conducted, or from the sale of microorganisms such as laboratory reagents, standards for analysis in laboratories, or intermediates to be used in the production of R&D substances, without being subject to TSCA § 5(a) notification requirements. Distribution of a microorganism to any person who will manufacture or process the microorganism for a commercial purpose or directly to "consumers" removes it from the category of R&D. Thus, so long as the microorganisms meet the requirements established at § 725.234, they would be eligible for the R&D small quantities exemption when sold solely for use as research products for experimentation on other substances, as intermediates for the manufacture of research chemicals, as analytical standards, and as quality control agents.

Contrary to one commenter's (#31) statements, not all commercial test kits are eligible for the R&D small quantities exemption. Test kits which are considered to be medical devices are subject to the FFDCA and therefore are not subject to TSCA, unless they have other uses subject to TSCA. In the case of test kits which are subject to TSCA jurisdiction, if intergeneric microorganisms are used in test kits to identify or quantify the presence of other substances, they may be eligible for the R&D small quantities exemption. Eligibility for the exemption depends on whether use of the test kit meets the conditions of § 725.234. Test kits which are distributed to

consumers are not eligible for the R&D small quantities exemption.

In addition to the requirements that an activity must involve "small quantities" of microorganisms manufactured solely for use in R&D, to qualify for this exemption, a manufacturer must comply with the notification requirements established at § 725.234(e) and explained in § 725.235(a) and (b). TSCA § 5(h)(3) requires manufacturers and processors to notify persons engaged in R&D of any risk to health that may be associated with the R&D microorganism; the form and manner of that notification, and the persons who must be notified are specified in §§ 725.234(e) and 725.235(b). Section 725.235(a) of the rule describes the information which the manufacturer must review to determine risks.

As to the commenter's (#31) request that EPA confirm the regulatory status of "microorganisms which are manufactured under prudent laboratory practices inside a laboratory in small quantities at laboratory scale," such microorganisms would be eligible for the § 5(h)(3) exemption only if they meet the requirements of §§ 725.234 and 725.235. Thus, to clarify for the commenter (#31), microorganisms manufactured, imported or processed in accordance with § 725.234, and "for commercial sale for research purposes," will be exempt from TERA reporting if they comply with the conditions in § 725.235.

3.Use of genetic libraries

Comments:

Two commenters (#38, 39) posed questions about the use of genetic libraries in the development of products that could potentially be subject to TSCA. Both commenters expressed concern about the amount of TSCA reporting that might be required because of the large number of microorganisms that make up a genetic library. One commenter (#38) was specifically concerned about the costs of complying with EPA's requirements. The other commenter (#39) recommended "that EPA adopt a simplified mechanism for regulating construction and commercial use of genetic libraries" and suggested a modified version of the Tier I exemption for categories of libraries with common properties.

EPA response:

EPA believes that most gene libraries subject to TSCA would likely be eligible for the R&D small quantities exemption. A gene library is a collection of a very large number of recombinants, each containing a fragment of another organism's genome which together contain the complete genome of the other organism. The DNA fragments which together represent the complete genome may be stored in bacteria, phage, or cosmids. An example of a library would be 4×10^5 lambda phages containing the human genome. In many cases, collections of microorganisms constituting a genetic library may be intergeneric. Gene libraries serve in R&D as the source of gene(s) desired by researchers or developers. The fragment containing a desired gene is selected from a member of the library and further processed to create the genetic material ultimately combined into the

recipient to create a microorganism with the desired trait(s). It is this latter microorganism that is generally either the product or used to create a product.

Gene libraries and their constituent members can be considered reagents, since fragments contained in them serve as the starting point for a construct which will ultimately be inserted into a microbial recipient to create the product microorganism. Therefore, constituents of gene libraries and gene libraries themselves which would be subject to TSCA jurisdiction but which are sold or used exclusively for R&D are eligible for the R&D small quantities exemption if they meet the requirements of §§ 725.234 and 725.235.

D.R&D IN CONTAINED STRUCTURES SUBJECT TO TSCA AND ANOTHER FEDERAL AUTHORITY

In the preamble to the proposed rule, EPA discussed situations where R&D activities might be subject to both TSCA and another Federal authority. EPA proposed different approaches to dealing with overlapping jurisdiction, depending on whether the R&D activities were conducted in a contained structure or involved intentional environmental testing.

EPA proposed a complete exemption from EPA-specific reporting under TSCA § 5(h)(4) for research on new microorganisms in contained structures, if the researcher is:

receiving research funds from another Federal agency which controls the research in accordance with applicable portions of the NIH "Guidelines for Research Involving Recombinant DNA Molecules." This control may be exercised through direct regulatory authority or through requiring compliance with the NIH Guidelines as a condition of receiving funds.

See proposed § 725.232(b), 59 FR 45526, 45574 (September 1, 1994).

In the proposed rule, EPA also discussed exempting from TSCA § 5 requirements the intentional environmental testing of new microorganisms, when another Federal agency has clear regulatory authority and EPA determines that the other Federal agency's review addresses criteria equivalent to those which would be evaluated under TSCA § 5. Specifically, EPA indicated that it was working with USDA/APHIS to develop an exemption from TSCA § 5 requirements for R&D field tests reviewed by APHIS under the Federal Plant Pest Act and the Plant Quarantine Act.

EPA received eleven comments (#7, 8, 18, 22, 23, 24, 28, 29, 30, 33 39) regarding R&D activities subject to TSCA and another Federal authority. Four of the commenters (#8, 18, 22,

39) discussed R&D in contained structures subject to another Federal agency. Seven of the commenters (#7, 18, 23, 28, 29, 30, 33) discussed Federal agency R&D subject to TERA reporting.

Comments:

Three commenters (#8, 18, 24) specifically indicated support for the proposal to exempt from EPA requirements those researchers who mandatorily comply with the NIH Guidelines. Four commenters (#8, 18, 22, 39) felt that researchers who voluntarily comply with the NIH Guidelines should also be exempt from the TSCA § 5(h)(3) requirements. One of these commenters (#18) indicated that:

[i]t appears discriminatory that funding from a Federal agency is a criterion for the R&D exemption, rather than compliance with the guidelines. After all, some institutions, e.g., colleges and non-profit institutions may not have outside funding at a particular time, but should be considered equal to all other parties if they demonstrate compliance to NIH guidelines.

Another commenter (#8) stated that any researchers required to comply with the Guidelines should be exempt. "This should include universities receiving federal funding, agencies and industry required to comply with the NIH Guidelines, e.g., by lease requirements by institutional, municipal or state ordinance."

EPA response:

All Federal agencies which support or conduct recombinant DNA (rDNA) research abide by the NIH Guidelines. Only research institutions that receive Federal funding for rDNA research are required through contract provisions to ensure that all rDNA research conducted at or sponsored by the institution, regardless of the source of funding, complies with the NIH Guidelines. Research conducted by persons who are following the NIH Guidelines for other reasons may not be subject to oversight by another Federal agency. For example, research conducted by a company which voluntarily chooses to follow the NIH Guidelines would not be subject to Federal oversight unless Federal funds were used in whole or in part in the conduct of the research. EPA's requirements at §§ 725.234 and 725.235 ensure that research subject to TSCA is required by law to be performed under appropriate conditions.

EPA has retained at § 725.232(b) its complete exemption from TSCA § 5 obligations for research on new microorganisms in contained structures, if the researcher is receiving funds from another Federal agency which requires compliance with the NIH Guidelines. This includes all research, whether directly funded by an agency or not, at a university or institution that adheres to the NIH Guidelines on an institution-wide basis as a condition of receiving Federal funds. EPA developed this exemption to avoid duplicative oversight with other Federal authorities. Researchers who are complying with the NIH Guidelines voluntarily or through vehicles, such as contracts or local regulations, will not be eligible for the exemption at § 725.232, because their research is not being overseen by another Federal agency. However, as discussed further below, EPA believes that anyone who is complying with the NIH Guidelines should be able to meet the requirements of §§ 725.234 and 725.235 with little difficulty.

In order for EPA to make the findings required by TSCA §§ 5(h)(3) and 5(h)(4) to exempt researchers from reporting requirements, EPA must ensure compliance with procedures designed

to prevent uncontrolled releases of microorganisms. These types of procedures are laid out in the NIH Guidelines and the companion "Biosafety in Microbiological and Biomedical Laboratories" (Ref. 23). EPA has provided an exemption at § 725.234 that is designed to provide complementary oversight of those research activities that are not required by contract law, as discussed above, to comply with the Guidelines. The provisions of § 725.234 effectively apply NIH Guidelines principles to those institutions, primarily commercial facilities, that are not legally required to abide by them. EPA also notes that the NIH Guidelines specifically apply to rDNA research. Not all intergeneric microorganisms will be constructed using rDNA techniques, and therefore not all researchers working with intergeneric microorganisms would be required to comply with the Guidelines. EPA regulations extend the benefits of the NIH Guidelines by effectively applying their principles to microorganisms other than those specifically mentioned in the Guidelines. Researchers subject to TSCA can meet the requirements of the R&D small quantities exemption by complying with the NIH Guidelines and keeping records of such compliance for R&D activities subject to TSCA. A detailed comparison of the NIH Guidelines and the requirements of the R&D small quantities exemption can be found in EPA's response in the next section of this Unit (see IV.E.1.).

E. REQUIREMENTS FOR SMALL QUANTITIES/CONTAINED R&D EXEMPTION

EPA indicated in the proposed rule that for those researchers who are voluntarily complying with, but are not subject to, the NIH Guidelines, the requirements of the R&D small quantities exemption at § 725.234 could be met by having the principal investigators (PIs) serve as the TQIs required by § 725.234(b) and keep records indicating that they abide by and are following the NIH Guidelines for the specific TSCA-subject R&D activities. EPA proposed to rely on the experience and judgement of the TQI to select containment and inactivation controls appropriate to the microorganism(s) being utilized. In some cases, the TQI could find it appropriate to use the NIH Guidelines, and in others, the TQI might not. EPA took this position because EPA recognized that many different kinds of microorganisms displaying a wide range of characteristics could potentially be used in research and that the type of controls appropriate for one microorganism might have limited relevance to other microorganisms.

EPA received 14 comments (#8, 15, 18, 22, 23, 24, 25, 26, 28, 30, 31, 33, 35, 39) requesting clarification of conditions of the R&D small quantities/contained structure exemption. The comments can be divided into two categories: (1) comments asking for clarification of use of the NIH Guidelines, and (2) comments asking for clarification of other aspects of the containment requirements.

1. Clarify use of NIH Guidelines

Comments:

Ten commenters (#8, 18, 22, 24, 25, 28, 30, 33, 35, 39) indicated support for use of the NIH Guidelines and requested clarification and/or made suggestions concerning the relationship of the

NIH Guidelines to the R&D small quantities exemption. One commenter (#18) also felt that EPA should require all researchers to use the Guidelines.

Two commenters (#8, 18) stated that the authorized official mentioned in § 725.234(d)(2) should be an IBC chair. One of these commenters (#18) also indicated that EPA should indicate which version of the NIH Guidelines the proposed rule was referring to and that EPA should allow for changes in the Guidelines. The other commenter (#8) noted that it was not clear who would pay the cost of EPA inspections and under what conditions the inspections would be conducted.

Five commenters (#25, 28, 33, 35, 39) stated that the recordkeeping requirements of the R&D small quantities exemption were too burdensome. Two commenters stated (#28, 33): "Unfortunately, the regulatory language in this section is confusing and implies significant recordkeeping unrelated to health or safety concerns. BIO believes that adherence to the NIH Guidelines as part of a company's standard operating procedure addresses the concerns set forth by EPA." These commenters proposed that the following language be incorporated in the final rule:

To demonstrate compliance with this part, a company needs to document and certify that it is following the NIH Guidelines. For experiments that are exempt from the NIH Guidelines, documentation of the exemption is sufficient. For those not exempt from the Guidelines, the appropriate Institutional Biosafety Review documentation is all that is required.

Another commenter (#39) urged EPA to allow oversight by principal investigators and IBCs to be "an explicit alternative to the requirements of § 725.234."

EPA response:

EPA disagrees with the commenter (#18) who believes that EPA should require all researchers to use the NIH Guidelines. While EPA considers the NIH Guidelines to provide the primary standard for laboratory research, EPA believes that it is appropriate to allow TQIs to have the option of relying on their experience and judgement in selecting appropriate containment as opposed to being forced to rely solely on the NIH Guidelines. In addition, not all TSCA-subject microorganisms will also be subject to the NIH Guidelines, since the Guidelines focus on rDNA molecules and EPA focuses on intergeneric microorganisms as "new". Therefore some researchers will need to rely for some activities on EPA's criteria at § 725.234, since their activities will not be covered by the NIH Guidelines. In structuring its approach, EPA believes it has provided an appropriate measure of flexibility to researchers. Additionally, EPA believes that those researchers who currently comply with the NIH Guidelines, but are not eligible for the exemption under § 725.232, nevertheless can comply with the requirements of §§ 725.234 and 725.235 with little additional burden beyond that imposed by the NIH Guidelines.

With respect to the requirement at § 725.234(d)(2) for certification by an authorized official, EPA recognized in the proposal (59 FR 45540 (September 1, 1994)) that IBCs and similar committees

are charged with assessing the containment selected by researchers. EPA encourages the active use of such committees and agrees that an authorized official may be an IBC chair. Regarding the comments about inspections, EPA inspections would be conducted under the authority of TSCA § 11 to assure compliance with the requirements of § 725.234.

EPA does not agree with the commenters who believe that record keeping for the R&D small quantities exemption is burdensome. As EPA noted in the proposal, EPA believes that persons following the NIH Guidelines would keep adequate records as part of normal procedures at an institution where IBCs are responsible for ensuring the safety of research. Such records are likely to be adequate for ensuring the safety of research. This issue is discussed in more detail below in a comparison of the NIH Guidelines and the requirements of §§ 725.234 and 725.235.

Several commenters suggested that EPA adopt the NIH Guidelines as a requirement for the R&D small quantities exemption. As discussed previously, EPA believes that it is more appropriate to show researchers how the use of the NIH Guidelines can fulfill the requirements of the R&D small quantities exemption. In general, EPA expects that companies currently complying with the NIH Guidelines will also be able to satisfy the requirements of the R&D small quantities exemption with little additional burden.

Comparison of NIH Guidelines and §§ 725.234 and 725.235. In the first section under IV. Roles and Responsibilities, the NIH Guidelines state:

The safe conduct of experiments involving recombinant DNA depends on the individual conducting such activities. The NIH Guidelines cannot anticipate every possible situation. Motivation and good judgment are the key essentials to protection of health and the environment.

The policy of the NIH Guidelines is similar to EPA's approach to the R&D small quantities exemption for microorganisms with its reliance on the experience and judgment of the technically qualified individual (TQI). EPA believes that researchers who are conducting research using intergeneric microorganisms in contained structures and who are voluntarily complying with the NIH Guidelines could easily meet the requirements of R&D small quantities exemption set forth in §§ 725.234 and 725.235. A review of the most recent complete version of the NIH Guidelines (Ref. 11) indicates that the responsibilities of the Principal Investigator (PI) listed in Section IV-

B-4 are similar to the requirements of the TQI under §§ 725.234 and 725.235. The following discussion is intended to assist researchers who wish to determine how compliance with the NIH Guidelines meets the specific requirements of §§ 725.234 and 725.235.

1. Section 725.234(a) requires that the microorganism be used solely for R&D activities. While there is not an equivalent PI responsibility in the NIH Guidelines, this requirement is compatible with the PI responsibilities set out in Section IV-B-4 of the NIH Guidelines discussed more specifically below. The Guidelines by implication refer primarily to research, the title of the Guidelines includes the term "research," and Section IV-B-4 specifies the PI's responsibilities for

various aspects of research activities.

2. Section 725.234(b) requires the microorganism to be used under the supervision of a TQI who must be qualified to comply with the requirements of § 725.234. Section IV-B-4 of the NIH Guidelines, which sets out the responsibilities of the PI, indicates that the PI is responsible for full compliance with the NIH Guidelines on behalf of the institution.

3. Section 725.234(c) requires that there be no intentional testing outside a contained structure. Section IV-B-4-e(4) of the NIH Guidelines charges the PI with ensuring the integrity of the physical containment and biological containment. The PI's selection of the appropriate containment level from either Appendix G, which specifies physical containment for standard laboratory experiments, or Appendix K, which specifies physical containment for large scale research or production uses of recombinant organisms, also ensures that there will be no intentional releases from the contained structure.

4. Section 725.234(d)(1) requires the TQI to select and use appropriate containment and/or inactivation controls. Under Section IV-B-4-c of the NIH Guidelines, the PI is responsible for making an initial determination of the required levels of physical and biological containment, selecting appropriate microbiological practices and laboratory techniques, and submitting research protocols to the IBC for review and approval.

5. Section 725.234(d)(2) requires the TQI to have the selection of containment and/or inactivation controls approved and certified by an authorized official of the institution. In many cases, compliance with the NIH Guidelines would satisfy this requirement. Under section IV-B-4-c-(3) of the NIH Guidelines, the PI must submit the research protocol to the IBC for review and approval. The IBC's approval of the protocol would satisfy the requirement at § 725.234(d)(2). In cases where the experiments are exempt from submission to the IBC under the NIH Guidelines, the PI should document such exemption and have this documentation approved by an authorized official, who does not necessarily need to be the IBC Chair. For example, the official could be the PI's supervisor or the institution's Biological Safety Officer (BSO). EPA believes that even where experiments are exempt from submission to the IBC, the PI would be required under the NIH Guidelines, at a minimum, adhere to standard microbiological practices such as those described in "Biosafety in Microbiological and Biomedical Laboratories" (Ref. 23).

6. Section 725.234(d)(3) requires the development of records describing the selection and use of the containment and/or inactivation controls as specified in § 725.235(c). Section IV-B-4-a-(6) of the NIH Guidelines indicates that the PI shall adhere to IBC-approved emergency plans for handling accidental spill and personnel contamination. Section IV-B-4-e-(3) states that the PI shall correct work errors and conditions that may result in the release of recombinant DNA materials. While Section IV-B-4-a does not specify record keeping responsibilities for the PI, EPA believes that submissions to the IBC required by Section IV-B-4-c, general documentation of routine standard operating procedures, and specific notation in laboratory notebooks will generally contain the information required under § 725.234(d)(3).

7. Section 725.234(e) requires the researcher to notify all persons in its employ, or to whom it directly distributes the microorganism, who are engaged in experimentation, research, or analysis on the microorganism, including the manufacture, processing, use, transport, storage, and disposal of the microorganism associated with research and development activities, of any risk to health identified under § 725.235(a). The notification must be made in accordance with § 725.235(b). Section 725.235(a)(1) provides that for research conducted in accordance with the NIH Guidelines, the manufacturer, importer, or processor must meet the conditions laid out at IV-B-4-d of the NIH Guidelines in making the determination of risk to health. Section 725.235(a)(2) lists the information which must be considered in making the determination of risk to health for research which does not qualify under subsection 235(a)(1).

Section 725.235(b)(1) requires the researcher to adequately notify the persons identified in § 725.234(e) of health risks determined under § 725.235(a). Section 725.235(b)(2) requires the researcher to provide written notification of the following when the microorganism is distributed to any persons not in the institution's employ: (1) that the microorganism is to be used solely for R&D, and (2) any health risks specified in § 725.235(b)(1).

Under Section IV-B-4-d of the NIH Guidelines, the PI is required to provide laboratory staff with the protocols describing potential biohazards and precautions to be taken: to instruct and train laboratory staff in safety practices and procedures for handling accidents, and to inform staff regarding precautionary medical practices advised or requested. While there is no responsibility for the PI directly equivalent to the requirements of § 725.235(a), in order to be able to carry out the responsibilities of Section IV-B-4-d, the PI would likely need to evaluate information similar to that described in § 725.235(a).

Under Section IV-B-4-a-(7), the PI shall comply with the Appendix H shipping requirements for recombinant DNA molecules. EPA interprets the requirement in section IV-B-4-a-(7) to notify laboratory staff broadly. To be eligible for the R&D small quantities exemption, institutions who have contractors conducting part of their research with intergeneric microorganisms subject to TSCA must ensure that the contractors also comply with the NIH Guidelines and the requirements of §§ 725.234 and 725.235.

Researchers should carefully read the requirements of § 725.234(e) and the related requirements in § 725.235 to ensure that they have correctly identified all persons who must be notified of potential risks and notified them appropriately.

8. Section 725.235(c) specifies the manner is which persons conducting research in accordance with the NIH Guidelines may satisfy record keeping requirements under these regulations. Such persons need only document that they have complied with the applicable provisions of the NIH Guidelines. Section 725.235(c)(2) requires researchers to keep the following records: a description of the selection and use of containment and/or inactivation controls for each

microorganism; certification by the authorized official; copies of information evaluated under § 725.235(a); documentation of the nature and method of notification under § 725.235(b)(1); and information about persons to whom the microorganism was distributed, the quantity distributed, and copies of notifications required under § 725.235(b)(2). As discussed above for § 725.234(d)(3), although section IV-B-4-a of the NIH Guidelines does not specify record keeping responsibilities for the PI, EPA believes that submissions to the IBC, general documentation of routine standard operating procedures, and specific notation in laboratory notebooks should contain much of the information required under § 725.235(c)(2).

The records required by these sections contain the information demonstrating the researcher's eligibility for the R&D small quantities exemption and would be subject to EPA review during any inspection. Therefore, researchers should review these requirements carefully to ensure that the appropriate records will be maintained during the course of the research.

Like the NIH Guidelines, EPA's regulations cannot anticipate every research situation. Therefore, using the above discussion as guidance, researchers subject to TSCA and complying with the NIH Guidelines should evaluate their specific research situation to determine whether their use of the Guidelines also fulfills the requirements of §§ 725.234 and 725.235.

2. Clarify containment requirements

Comments:

Four commenters (#15, 23, 26, 31) asked that EPA clarify certain aspects of the containment requirements for the R&D small quantities exemption at § 725.234. One commenter (#31) did not feel that EPA's requirements for selection and use of containment and/or inactivation controls inside a structure adequately recognized the "inherent variability" in basic biotechnology research. This commenter recommended that the containment and/or inactivation controls be selected "based on an assessment of the range of possible outcomes from the experimentation."

Another commenter (#26) felt that EPA should clarify "the relationship between containment/leaks and the risk of the organism." This commenter suggested that contained structures "be defined as those which provide primary containment as discussed in § 725.234 and secondary containment measures at areas defined from the abovementioned hazard/release assessment." This commenter also wanted EPA to include an explicit requirement for a hazard/release assessment and to provide guidance on acceptable levels of probability of establishment.

One commenter (#23) expressed support for EPA's containment requirements but felt that it was necessary for EPA to formally clarify that bioreactors could be eligible for the R&D small quantities exemption.

EPA received two comments (#15, 23) relating to the role of the technically qualified individual

(TQI). One commenter (#23) felt that it was appropriate to give the TQI a fair amount of leeway in selecting containment because of the variability in microorganisms that could be used. The other commenter (#15) believed that EPA should play a stronger role in oversight of TQIs. This commenter suggested that EPA modify its approach by planning to conduct routine inspections and to routinely review TQI records.

EPA response:

EPA disagrees with the commenter (#31) who believes that EPA did not adequately consider the "inherent variability" in basic biotechnology research. In fact, it is because EPA recognizes that many different kinds of microorganisms displaying a wide range of characteristics could potentially be used in research that EPA is not prescribing rigid containment requirements. EPA is relying on the experience and judgement of the TQI to select appropriate containment and is expecting that most researchers will rely on the NIH Guidelines, which EPA considers to provide the primary standard for laboratory research. This is also why EPA does not believe that it is necessary to formally require a risk assessment or provide additional containment guidance as suggested by one commenter (#26). EPA expects the TQI to consider the potential risk of the microorganism(s) and of the experiment and, applying the NIH Guidelines and other appropriate guidance as well as expert judgement, select the appropriate controls on the experiment. When research projects involve multiple organisms, EPA expects the TQI to select conditions that would be appropriate for handling all of the organisms.

EPA agrees with the commenter (#23) who stated that it was appropriate to give the TQI leeway in selecting containment. As stated earlier, EPA is aware that a variety of microorganisms will be used in research that could be subject to TSCA oversight. EPA also recognizes that the type of controls appropriate for one microorganism might have limited relevance to other microorganisms. The TQI should possess the necessary experience and judgement to select appropriate containment. This assumption is consistent with the NIH Guidelines' reliance on the oversight of the Principal Investigator. Additionally, the TQI's decisions must be certified by an authorized official. Therefore, EPA does not believe that EPA needs to strengthen its oversight of TQIs.

With regard to the definition of "contained structure," EPA intentionally chose a broad definition which could encompass a variety of structures, such as pilot fermentation facilities, greenhouses, laboratories, and bioreactors, if these structures can meet the other requirements of the exemption. EPA disagrees with the commenter (#23) who believed it is necessary for EPA to further define "contained structure." EPA believes further definitions of "contained structure" are not necessary, because the TQI's selection of containment and inactivation controls appropriate to the microorganism being used should be the primary means of ensuring that a structure is "contained."

F.EXEMPTIONS FROM TERA REPORTING FOR CERTAIN R&D ACTIVITIES

CONDUCTED OUTSIDE A STRUCTURE

In the proposed rule, EPA discussed a process for exempting small-scale field tests of certain microorganisms from TERA reporting. The TERA process is discussed in Unit G of this document. To qualify for the exemption, certain criteria regarding the recipient microorganisms, the source(s) and characteristics of the introduced genetic material, and the conditions of use would need to be met. EPA proposed certain strains of *Bradyrhizobium japonicum* (*B. japonicum*) and *Rhizobium meliloti* (*R. meliloti*) as candidates for exemption from TERA reporting, based on EPA reviews of PMNs voluntarily submitted for these microorganisms under the 1986 Policy Statement and field test data generated in these field trials.

1. Exemptions for Certain Nitrogen-Fixing Bacteria

Comments:

EPA received ten comments (#5, 8, 12, 15, 17, 18, 23, 27, 30, 36) on its proposed TERA exemption for certain intergeneric strains of the nitrogen fixing bacteria,*R. meliloti* and *B. japonicum*. Six commenters (#8, 12, 18, 23, 30, 36) supported the proposed exemptions with some modifications. Three commenters (#5, 15, 27) opposed the exemptions. One commenter (#17) submitted journal articles to support his position that EPA should proceed with caution when considering the two exemptions.

Two commenters (#23, 36) supported the exemptions as proposed and encouraged EPA to broaden the exemption opportunities. Three commenters (#8, 18, 30), while expressing support for the exemption, also indicated their support for a limited notification provision.

Two commenters (#8, 18) recommended that the strains of *R. meliloti* and *B. japonicum* proposed for exemption be limited to those that do not produce rhizobitoxin, that the exemption be expanded to allow the use of markers other than antibiotic resistance markers, and that a monitoring provision be included. Two commenters (#12, 15) believed that it was necessary for EPA to strengthen its requirements for the technically qualified individual (TQI) to limit exposure.

Two commenters (#5, 15) opposed the TERA exemptions based on EPA's general lack of experience with genetically modified microorganisms. Four commenters (#5, 12, 15, 27) specifically opposed EPA's proposal to allow use of antibiotic resistance markers in the microorganisms eligible for the TERA exemption.

Additionally, commenters raised objections to requirements for the TERA itself and the TERA exemption that State and local authorities be notified of planned testing. These comments are discussed in more detail in Unit V.F. of this document.

EPA response:

EPA agrees with commenters who suggest that a limited notification procedure, with fewer requirements than a TERA, is appropriate for certain field tests under certain conditions. EPA has established such a procedure in § 725.238 for the exemption from TERA reporting for certain activities conducted outside a contained structure. If a field test qualifies for this exemption from full TERA reporting, then prior to initiating their field tests submitters must send a notice to EPA. The notice must include the location and the estimated start date of the test as well as certification of compliance with the specific conditions of the exemption.

In response to comments, EPA has modified some of the specific conditions of the exemption for certain strains of *R. meliloti* and *B. japonicum*. EPA has determined that it will follow a conservative course and limit the source of genetic modifications to genes from the closely related microorganisms *Rhizobium* and *Bradyrhizobium* and, as discussed below, to incorporation of an antibiotic resistance marker which EPA has previously reviewed. EPA disagrees with commenters who do not believe that sufficient field test data have been generated on these microorganisms. There is extensive information on *R. meliloti* and *B. japonicum* generally documenting the lack of adverse effects on human health and the environment. EPA has reviewed field test data from several experiments using these organisms. As noted in the proposal, EPA incorporated the public dockets pertaining to the reviews of *R. meliloti* (18 strains: P87-568 through 570, P88-1115 through 1122, P89-280, P90-339, and P92-399 through 403) and *B. japonicum* (six strains: P88-1275 through 1278, and P89-340 and 341), along with the field test data, into the docket for this rulemaking. These field tests have demonstrated that the strains modified by the addition of genetic material from other rhizobia and by addition of antibiotic resistance markers are similar to the unmodified parental strains in colonization, survival, nodulation and effects on plant growth. In addition, EPA has evaluated general and specific information in the open scientific literature concerning these species, and subcommittees of EPA's Biotechnology Science Advisory Committee (BSAC) were convened to discuss general and specific issues associated with the proposed R&D field tests with these species. For these reasons, EPA believes the proposed exemptions to be appropriate and disagrees with commenters who do not believe that sufficient field data have been generated on these microorganisms.

Two commenters recommended limiting exemption to those *Bradyrhizobium* strains that do not produce rhizobitoxine. Rhizobitoxine is a phytotoxine, 2-amino-4(2-amino-3-hydroxypropoxy)-trans-but-3-enoic acid, that may be produced by bradyrhizobia in nodules. As mentioned by commenters, this toxin can cause chlorosis (yellows) in susceptible cultivars. It is not uncommon for bradyrhizobial isolates to produce this toxin. EPA concurs that if such isolates were to become commercial products, significant loss in yield of soybeans might occur. However, EPA has not limited the exemption for *Bradyrhizobium* to non-rhizobitoxine producing strains for several reasons. First, the TERA exemption pertains to small scale field testing of ten (10) acres or less. Should a rhizobial strain express rhizobitoxine, the effects would be noticed early in product development, e.g. at the research stage. It is unlikely that a strain causing chlorosis would be developed into a commercial product. There would be a disincentive for a submitter to

knowingly utilize a toxin producing strain as a recipient, since a submitter is unlikely to choose to develop a product that reduced, rather than increased, yield. The use of rhizobitoxine producing strains is thus likely to be self-limiting. Moreover, gene transfer from the test organism to other rhizobia or other microorganisms is not expected to present a problem because of the common occurrence of the rhizobitoxine gene in naturally occurring isolates. These naturally occurring isolates, because they are likely to be present in relatively high numbers in soil, would be far more likely to serve as a source for transfer of the rhizobitoxine gene to soil microorganisms than would a test organism under controlled small scale study. In any event, commercialization of a rhizobitoxine-producing strain would be contingent on MCAN notification to and review by EPA, and the issue would be evaluated at that time.

EPA welcomes the observations provided by one commenter (#17) that intrinsic antibiotic resistance is commonplace in *B. japonicum* and that *Bradyrhizobium* can persist after addition to soils. These documented observations coincide with data obtained by EPA of field experimentation using intergeneric *Bradyrhizobium*. Similarly, in developing this exemption, EPA considered the information that the transfer rate of transmissible antibiotic resistance elements between *Bradyrhizobium* and the related *Agrobacterium tumefaciens* was very low ($<10^{-8}$ per recipient), although transfer was not routinely reproducible.

While EPA concurs with the scientific observations made by the commenter, EPA does not agree with the regulatory conclusions drawn by this commenter. TSCA is a risk balancing statute, not one that demands absolute absence of risk. The exempted use of the named species in § 725.239 permits only field testing on 10 acres or less of land. As noted above, this exemption is based on individual experiments reviewed and previously approved by EPA. The reviews of these experiments considered information of the kind supplied by the commenter. The conclusions of these reviews were that the experiments could go forward, because the benefits outweighed the potential risks. The information supplied by the commenter, especially the reports of very low rates of gene transfer under optimal laboratory conditions, provides no evidence that would serve to contradict EPA's conclusions based on its experience with the prior field releases serving as the basis of the TERA exemption.

With regard to the issues raised by commenters (#5, 12, 15, 27) concerning the appropriateness of considering eligible for exemption from TERA reporting certain intergeneric strains of *B. japonicum* or *R. meliloti* constructed using antibiotic resistance genes from any source, EPA has determined it will follow a more conservative course than originally set forth in the proposed rule. Analysis of natural isolates of rhizobia show that these microorganisms exhibit high levels of intrinsic antibiotic resistances. Indeed, the high frequency of a large variety of antibiotic resistance genes in rhizobia, on occasion, render these genes of dubious value as markers. The high level and variety of genes encoding antibiotic resistance in rhizobial populations, coupled with the limitations on exposure imposed by § 725.239 suggest that use of antibiotic markers in *B. japonicum* and *R. meliloti* under the conditions of § 725.239 would present overall a low probability of presenting an unreasonable risk.

Nonetheless, EPA has determined that for the exemption described at § 725.239, it will follow the more conservative course of allowing use in *B. japonicum* and *R. meliloti* of only the antibiotic resistance marker EPA has previously reviewed for use in these microorganisms. The regulatory text at §§ 725.239(a)(2)(ii)(A)(1) and 725.239(b)(2)(ii)(A)(1) has been modified to read as follows:

For structural genes encoding marker sequences, the gene is limited to the *aadH* gene, which confers resistance to the antibiotics streptomycin and spectinomycin.

Based on EPA's analysis of use of this marker in rhizobia, and including consideration of the advice of the January 4, 1995 BSAC Subcommittee, the use of streptomycin and/or spectinomycin resistance markers in *B. japonicum* and *R. meliloti* currently meets this requirement of the exemption.

EPA recognizes that the exemption at § 725.239 is narrow and may only apply to very few research projects. It may be the case in the early years of the TERA program that TERA exemptions are narrowly written to apply to specific microorganisms that have completed TERA review. However, EPA hopes that in the longer term as EPA gains greater experience reviewing intergeneric microorganisms for environmental uses, broader exemptions can be written. To that end, EPA has placed general requirements for the TERA exemption in § 725.238 and will use § 725.239 to list certain microorganisms for the exemption and the specific conditions of use as needed.

2.Federal agency R&D subject to TERA reporting

Comments:

EPA received seven comments (#7, 18, 23, 28, 29, 30, 33) on its proposals for addressing field testing subject to more than one agency's jurisdiction. Five commenters (#7, 18, 23, 29, 30) specifically supported EPA's discussion of potentially deferring to other agencies' reviews and determinations when appropriate. Three commenters (#18, 23, 30) indicated that EPA should specifically clarify EPA's relationship with USDA/APHIS. One of the three commenters (#30) also stated that the relationship to state regulation is unclear, particularly for states which have their own biotechnology regulations.

Four commenters (#18, 23, 28, 33) suggested extension of EPA's proposal to defer to other Federal agencies. One commenter (#18) suggested that "in addition to specifically recognizing those agencies [which already have jurisdiction over a product], previously developed protocols applicable to microorganisms which could be covered by TSCA should be mentioned. Another commenter (#23) urged "EPA to return to the concept expressed in the 1991 draft proposed rule that this exemption would be made available to noncontained R&D as well as contained, by having EPA enter into memoranda of understanding with other federal agencies to resolve overlapping jurisdiction." Two other commenters (#28, 33) suggested that "EPA and NIH should

enter into a memorandum of understanding that OPPT [EPA] will have oversight of all field experimentation that would normally be covered by TSCA." These commenters also suggested that EPA place language in § 725.232 allowing EPA to review research that doesn't meet the criteria of § 725.232(a).

EPA response:

EPA agrees in principle with commenters who state that, when consistent with the requirements of the statutes involved, products subject to another statute as well as to TSCA need only be regulated by one of those agencies. Presently, EPA has identified the Plant Pest Act and Plant Quarantine Act administered by USDA/APHIS as presenting some degree of overlapping jurisdiction with TSCA for microorganisms. At this time EPA and USDA do not know of any products subject to overlapping jurisdiction. Should such situations arise, EPA will work with APHIS to develop a proposed exemption from TSCA § 5 requirements for R&D field tests subject to overlapping jurisdiction. In the future, should other cases of duplicative oversight between EPA and other Federal agencies arise, EPA will work with the other agencies involved to develop an appropriate solution. Regarding EPA's relationship to the states, EPA's coordination with the states is discussed in Unit V.F. of this document.

G.TERA REPORTING PROCESS

Under § 5(h)(4), EPA proposed to conditionally exempt from MCAN notification certain R&D activities involving new microorganisms. The exemption is conditional, since researchers must submit a TSCA Experimental Release Application (TERA), an abbreviated notification. Due to the availability of other exemptions for R&D activities under these rules, EPA expects that the TERA will be used primarily for environmental research. In the proposed rule, EPA indicated that its goal was to review TERAs in 60 days, but that for good cause, EPA could extend the initial TERA review period by an additional 60 days, for a total of 120 days.

Comments:

EPA received six comments (#8, 15, 18, 23, 24, 26) on the TERA process. Three commenters (#15, 23, 26) supported the expedited TERA process for review of field tests. Two commenters (#8, 18) opposed the use of the TERA process. One commenter (#24) asked for clarification of the process.

Two commenters (#8, 18), believing that the time and expense of TERA preparation was not justified, suggested an alternative that would have a researcher's Institutional Biosafety Committee (IBC) forward a proposed experiment to EPA for a 30-day review. While these commenters stated that the information requirements for test site and target organisms were too extensive and open-ended, they stated that because monitoring data were important for

commercialization, the requirement for monitoring data should include information on type, frequency, and duration of monitoring.

One commenter (#24) requested that EPA clarify the TERA process, especially the scientific reasons EPA would rely on for extending the review period beyond 60 days. This commenter also felt that EPA should publish the outcome of TERA reviews and include a discussion of issues considered, because the commenter was concerned that there could be delays since EPA had not specified information it would need for its review.

Additionally, commenters raised objections to requirements for the TERA itself and the TERA exemption. They also requested that State and local authorities be notified of planned testing. These comments are discussed in more detail in Unit V.F.

EPA response:

EPA believes that it is necessary to establish a review and approval process specifically for R&D activities involving environmental release. While many field tests of new microorganisms will be determined to pose low risks, this assumption cannot be made for field tests in general, and thus EPA finds some type of review is warranted. However, EPA recognizes that full MCAN reporting also may not be warranted. Therefore, EPA has chosen to develop a review and approval process specifically tailored to address R&D.

EPA believes that the information requirements proposed for the TERA are appropriate. EPA must have sufficient information to evaluate the health and environmental effects of a planned field test. However, because a variety of microorganisms are potentially subject to TSCA, the requirements indicated in § 725.255 are necessarily broad. Not all of the requirements are equally applicable to all microorganisms. Submitters are encouraged to consult with EPA prior to preparing TERAs, so that appropriate information needs and concerns may be identified.

EPA has made minor changes to the regulatory text at § 725.270 to clarify that EPA is approving or denying the TERA. Therefore, the term “TERA agreement” which was used in the proposed rule has been changed to “TERA approval.” In addition to approving or denying the TERA, EPA may provide, in the TERA approval, conditions under which the R&D activity described in the TERA must be conducted in order for EPA to make the TSCA § 5(h)(4) finding that the R&D activity will not present an unreasonable risk to health or the environment.

As indicated in the proposal, in some circumstances EPA may extend the TERA review period in order to complete its review. For example, the review period might be extended if the submitter is asked to supply additional information. Other instances presenting a need for extensions include a Federal Advisory Committee Act (FACA) meeting of an expert science advisory group or the need to coordinate review with other Federal agencies.

H.OPTIONS FOR OVERSIGHT OF R&D ACTIVITIES

EPA proposed an approach for oversight of R&D activities which included a variety of exemptions from the full 90-day reporting process required for general commercial use activities. These exemptions have been discussed in Units IV.C. through G. of this document. EPA's goal was to provide a flexible process which tailored oversight to the level of risk. EPA asked for comment on its R&D exemptions, all of which have been discussed in this Unit, and indicated that the public could suggest other options for consideration. EPA received six general comments (#8, 18, 24, 25, 30, 33) on the proposed option for oversight of R&D activities. EPA also received seven comments (#8, 12, 15, 18, 23, 24, 33) on its specific alternative approach for low risk field tests.

1.General comments

Comments:

Six comments (#8, 18, 24, 25, 30, 33) were received on the proposed approach for oversight of R&D activities. Two commenters (#8, 30), while indicating that the R&D exemptions were comprehensible, did not believe that level of oversight correlated to level of risk. One of the two commenters (#30) suggested that EPA consider a notification based on performance standards, believing that "this would also eliminate the use of Federal funding as a determining factor in deciding if a R&D exemption should be allowed." The other of the two commenters (#8) stated that "it is erroneous to assume that self-sustaining or multiplying populations of microorganisms will do damage...[or] to presume that the microorganisms used in small-scale field tests will establish themselves permanently in the environment."

One commenter (#24), in noting that EPA planned to supplement the scientific expertise of its staff with additional experts when necessary, encouraged EPA to consider consulting with experts from private industry and offered to assist EPA in identifying those experts.

Several commenters (#8, 18, 25, 33) were concerned about other aspects of EPA's approach to oversight of R&D activities involving environmental release. One of these commenters (#33) indicated that there is sufficient information on small scale testing of microorganisms to indicate limited movement from the test site and suggested, therefore, that EPA use TSCA § 8 to develop a postcard notification process, rather than a § 5 review process. As proposed by this commenter, under TSCA § 8, EPA would require that 30 days prior to initiating a field test, researchers submit the following information:

A.The common and scientific name of the microorganism and a description of its known genetic characteristics;

B.The purpose, location, and date of the proposed field test;

C.The total amount of the microorganism or microorganism- containing product to be used during the field test;

D.An assessment, based on literature information and/or lab, greenhouse or growth chamber results, of the microorganism's ability to survive under conditions of the field test, along with any physical, chemical, or biological measures which will be used or available if there is a need to control the microorganism;

E.Information in possession of the applicant or publicly available regarding the potential environmental or health effects of such a field test;

F.The name of the principal investigator, the number of individuals that may be exposed during the field test and the duration of that exposure.

The commenter also suggested that EPA could utilize its authority under TSCA §§ 7 and 11, in conjunction with its § 8 authorities to provide, if necessary, a higher level of EPA oversight than § 8 alone.

Two commenters (#8, 18) recommended that EPA rely on certain publications on field testing microorganisms, specifically "the USDA's ABRAC document (Guidelines for Research Involving Planned Introduction into the Environment of Genetically-Modified Organisms); the book, "Microbial Ecology: Principles, Methods, and Applications", edited by M. A. Levin, R.J. Seidler, and Rogul, McGraw Hill, 1992, and the review by M. Wilson and S. Lindow. 1993, “Release of recombinant microorganisms”, Ann.Rev.Microbiol. 47:913-944.

Three commenters (#8, 18, 25) indicated that "since the TERA burden is structured to be minimal, the value of exemptions will rely primarily on the response time by EPA to exemption requests." One commenter (#18) also indicated that EPA consider developing exempt categories for small scale field tests involving "microorganisms with deletions or markers conveying no phenotypic trait of consequence to humans or the environment."

EPA response:

EPA disagrees with comments that the level of oversight imposed in its R&D exemptions is not correlated to level of risk. EPA discusses the basis for oversight of "new microorganisms” in Unit II.D. of this document. EPA has chosen to implement its R&D oversight in a manner which distinguishes between R&D activities in contained structures and R&D activities involving intentional release to the environment because of the greater overall potential in the latter case for survival, dissemination, and exposure to the microorganisms.

Within this broad structure, EPA has developed several exemptions which recognize the differing risk potentials presented by different settings and organisms. Two low burden exemptions have been developed for contained structure R&D which recognize the limited exposure and risk

potential of R&D which meets the contained structure conditions. The small quantities R&D exemption for microorganisms used in contained structures should not be overly burdensome, because EPA believes, as discussed previously in this Unit, that in most cases, researchers who are voluntarily complying with the NIH Guidelines will possess the information needed to meet the requirements at §§ 725.234 and 725.235. In any case, EPA expects that laboratory notebooks and other records normally kept in the course of research would contain most of the information required for compliance with the exemption.

The exemption for contained R&D which is also subject to another Federal authority is a complete exemption from TSCA § 5 obligations. EPA developed this exemption because persons conducting research subject to another Federal agency's requirements would already to subject to oversight. Researchers subject to TSCA who do not meet this criterion may still perform their R&D in accordance with the NIH Guidelines but must also meet the requirements imposed under these regulations. These contained structure R&D exemptions, which are discussed above in Unit IV.C. through E, are expected to be broadly applicable and are expected to minimize the occurrence of TERA reporting for contained structure R&D.

Another low burden exemption has been developed for R&D field trials which meet certain conditions and use specified microorganisms. The basis for this exemption is found in EPA's prior experience in reviewing microorganisms meeting these requirements and sets the stage for EPA to create additional exemptions at § 725.239 as EPA's experience grows (see Unit IV.F.). In addition, EPA is developing and plans to propose an exemption for low risk field trials. This exemption, once fully developed, is expected to be similar to the description in Unit II.D.5.2. of the proposed rule preamble (see Unit IV.H.2. of this document). Thus, when the full set of exemptions are developed, researchers will have a wide array of alternatives to the submission of a TERA, which is itself an exemption from full MCAN reporting (see Unit IV.G. of this document). The TERA consists of an abbreviated notice and a shortened review period relative to the MCAN.

EPA does not agree with the commenter (#33) who stated that there is sufficient information to indicate limited movement of microorganisms from small scale field tests. While limited movement may be associated with certain microorganisms under certain conditions, this assumption cannot be applied generally to the variety of microorganisms in a variety of uses which could potentially be subject to TSCA.

EPA also disagrees with the commenter (#33) who stated that a TSCA § 8 reporting rule would be appropriate for oversight of new microorganisms. Section 5 explicitly requires all manufacturers and processors to submit a notice to EPA 90 days before manufacturing new microorganisms, to permit EPA to review them to determine whether such new substances may present an unreasonable risk of injury to human health or the environment. EPA can only forego that review if it can determine in advance of such review that the substance will not present an

unreasonable risk of injury to health or the environment. Given that § 8(a) exempts small manufacturers and processors from coverage, EPA could not meet its § 5 obligations by issuing a regulation under § 8. EPA does not believe that sufficient information currently exists to determine that all small scale field testing for all intergeneric microorganisms currently or subsequently produced by small manufacturers and processors will not present an unreasonable risk of injury to health or the environment. EPA also lacks sufficient information to justify decreasing EPA's review period from 90 days to 30 days for all small scale field testing for all intergeneric microorganisms currently or subsequently produced, which are potentially subject to TSCA.

Nor would reliance on EPA's authority under §§ 7 and 11 compensate for the defect discussed above. Section 7 was designed as a response mechanism for risks that develop from the use of chemicals already in commerce. The purpose of § 5 review is to determine the health and environmental effects of a substance before commercial production begins, and to prevent such risks from developing by establishing restrictions which mitigate the risks without imposing undue economic burden. See, e.g., H.R. Conf. Rep. No. 1679, 94th Cong., 2d Sess. 65 (1976). The difference between the two mechanisms is particularly important in this context, since EPA's ability to control or address any resulting risks, should a "risky" intergeneric microorganism become established in the environment, would be limited at best.

In the 1986 Policy Statement, EPA discussed using TSCA § 8(a) to obtain information on a greater variety of microorganisms to assess whether additional categories of microorganisms not then subject to TSCA § 5 reporting might need to be regulated. Since that time, EPA has determined that it is focussing its TSCA § 5 authority on the appropriate categories of microorganisms and does not believe that a TSCA § 8 reporting rule for microorganisms would be useful. The type of information the commenter suggests that EPA should require in a § 8 rule is the type of information which EPA asks that researchers submit in the form of the TERA. EPA believes that the TERA reporting process has been appropriately established under TSCA § 5(h)(4). Further, EPA believes that TSCA § 5(h)(4) is the appropriate vehicle under which to refine its reporting program for microorganisms.

2.<u>Specific alternative for low risk field tests</u>

In the proposal, EPA briefly discussed an alternative exemption for certain R&D releases. This alternative would contain requirements for documentation and record keeping by a TQI and certification by an authorized official. Following the TQI's determination that the field test met the eligibility requirements, a notice summarizing the new microorganism and the proposed field test would be submitted to EPA for a 45 day review. In lieu of a TQI, the analysis could be performed by a third party review group such as an Institutional Biosafety Committee (IBC) as described in the NIH Guidelines.

<u>Comments</u>:

EPA received seven comments (#8, 12, 15, 18, 23, 24, 33) on its proposed alternative for low risk field tests. Five commenters (#8, 18, 23, 24, 33) supported the alternative. Two commenters (#12, 15) opposed the alternative.

One commenter (#23) indicated that "some form of self-certification, subject to EPA notification, would be appropriate for a variety of potential field tests of intergeneric microorganisms." Two other commenters (#8, 18), however, while supporting the alternative, indicated that "it is not clear how this would work in practice, since the TQI apparently must still notify EPA." Another commenter (#24) suggested that EPA provide a list, updated quarterly, of microorganisms that specifically meet the criteria. This commenter also stated that "EPA should also publish a listing of all microorganisms and genetic material that would now fall into this category prior to implementation of these rules." One commenter (#33), while expressing support for the alternative, strongly objected to the provisions requiring officials to accept full liability for harmful consequences of the field test.

One of the commenters (#12) opposing the alternative, indicated that it was premature because there is a lack of data on use of genetically modified microorganisms in the environment and "no consensus on what releases constitute low risk." The other commenter (#15) opposing the alternative was concerned that it represented an over-reliance on self-regulation by researchers and that the complexity of the determination involved would result in inconsistent decisions about risk.

EPA response:

EPA is not finalizing this option at this time. However, EPA intends to repropose an exemption along these lines at a later date to allow the public an opportunity to comment on the information on which EPA would rely to support the exemption.

V. OTHER ISSUES

A. MICROORGANISM - DEFINITION

EPA proposed to define "microorganisms" as those organisms classified under the 5-kingdom system of Whittaker (cited in Atlas and Bartha (1987)(Ref. 24)) in the kingdoms Monera (or Procaryotae), Protista, and Fungi, the Chlorophyta and the Rhodophyta of the Plantae, and viruses and virus-like particles. Therefore, this definition includes, but is not limited to, bacteria, protozoa, fungi, mycoplasmas, mycoplasma-like organisms, spiroplasmas, microphytoplanktons, green and red algae, viruses, and virus-like particles (e.g., viroids, satellites, and virusoids). Should new categories of organisms within the Monera, Protista, Fungi and the Chlorophyta and Rhodophyta of the Plantae be identified, these would also be considered microorganisms under

this definition.

EPA proposed to treat viruses of other microorganisms (also termed phages) as MGEs. EPA's MGE policy is discussed above in Unit II.D. In the proposed rule preamble, EPA indicated that it was not able to identify uses of viruses of macroorganisms that might be subject to TSCA. EPA asked if it was appropriate to apply the intergeneric interpretation to viruses of macroorganisms if TSCA uses for such viruses were identified.

Comments:

EPA received four comments (#8, 12, 18, 24) related to its definition of "microorganism." Three of the commenters (#8, 18, 24) indicated that they believed the definition to be reasonable. Two of the three (#8, 18) indicated that the more practical definition was "if the individual organism cannot be seen with the naked eye, it is a microorganism." These two commenters also stated that including members of the two plant groups was reasonable.

The four commenters (#8, 12, 18, 24) discussed EPA's proposed coverage of viruses. One of the commenters (#24) felt that the inclusion of viruses in the definition was reasonable. Two of the commenters (#8, 18) felt that the approach treating phages as MGEs was reasonable, although one commenter (#18) indicated that "The question should be asked, however, whether all phage should be included." A fourth commenter (#12) stated that because of the special risks posed by viruses, EPA should not apply the intergeneric approach to viruses of macroorganisms. This commenter also urged EPA to promulgate a Significant New Use Rule (SNUR) for TSCA uses of all deliberately modified viruses of macroorganisms.

EPA response:

EPA will retain the definition of "microorganism" as discussed in the proposed rule. Those who commented on the definition thought it was reasonable and included the appropriate organisms. EPA will treat phages as MGEs. A phage modified to contain genetic material from an organism classified in a different genus from the genus from which the modified (recipient) phage was isolated would be considered intergeneric. Under this interpretation a phage which has been modified to contain genetic material from a virus of a macroorganism would also be considered a "new" microorganism. No commenters identified current or imminent TSCA uses of viruses of macroorganisms. Therefore, EPA believes the best use of limited resources would be to develop an approach under TSCA for viruses of macroorganisms in the future if TSCA uses are identified. Anyone planning to use a virus of a macroorganism for a TSCA use should contact EPA regarding the status of the virus under TSCA § 5.

B.TSCA INVENTORY

EPA described in the proposed rule preamble how it planned to explicitly list microorganisms on the TSCA Inventory and the rationale for the proposed listing (59 FR 4555152, September 1,

1994). EPA proposed to identify microorganisms on the Inventory using a taxonomic designation and a consistent set of supplemental information on phenotypic and genotypic traits necessary to identify the microorganism as precisely as possible. Additionally, EPA indicated that it was considering requiring that microorganisms listed on the Inventory be deposited in a recognized culture collection.

In the proposed rule, EPA advised manufacturers and importers of any of the 192 microorganisms reported in 1978 to the initial TSCA Inventory that EPA planned to remove from the Inventory the explicit listing of these microorganisms. EPA believed that most of these microorganisms are not intergeneric; therefore they would be implicitly included on the Inventory and do not need to be explicitly listed. EPA asked manufacturers and importers of these microorganisms to inform EPA if any of the microorganisms were intergeneric and should not be removed from the Inventory.

EPA received 13 comments (#4, 8, 18, 23, 24, 25, 26, 28, 31, 33, 34, 35, 39) regarding Inventory issues. Commenters raised concerns about the following issues: (1) the culture collection requirement, (2) Inventory listing, (3) a "grandfather" period, and (4) microorganisms currently listed on the Inventory.

1.Culture collection requirement

Comments:

Eight commenters (#8, 18, 23, 24, 28, 33, 34, 35) expressed concern about EPA's proposal to require that microorganisms listed on the Inventory be deposited in a recognized culture collection. One commenter was concerned about the effect of EPA's requirement on patent protection. Two commenters (#8, 18) stated that such a requirement would be unnecessary and onerous at the R&D stage. Five commenters (#23, 28, 33, 34, 35) strongly opposed any requirement for deposit of a microorganism in a culture collection.

EPA response:

EPA has considered the concerns raised by commenters who oppose the culture collection requirement and has decided that deposit of new microorganisms in recognized culture collections is not necessary at this time for TSCA § 5 purposes. Some commenters (#23, 24) noted that a requirement for deposit in a culture collection would duplicate requirements under U.S. and international patent law. Several commenters (#8, 18, 24) also cited the costs associated with maintaining microbial cultures in culture collections. Concerns were also expressed by several commenters (#23, 24, 28, 33) regarding the need to establish procedures to maintain the confidentiality of microorganisms submitted to culture collections during the R&D stage and prior to issuance of a patent. It was not EPA’s intention to require that microorganisms being used for R&D purposes be deposited in a culture collection. EPA stated in the proposed rule that it was

"considering requiring that microorganisms listed on the Inventory be deposited in a recognized culture collection", 59 FR 45526, 45551 (September 1, 1994)(emphasis added); microorganisms being used for R&D are not eligible for inclusion on the Inventory. Nonetheless, EPA believes that several practical considerations were raised in the public comments. In addition, should EPA need to obtain a sample of a new microorganism, other avenues for doing so would be available to the Agency. Therefore, EPA has not made deposition of new microorganisms in a culture collection a requirement in this final rule.

2.Inventory listing

Comments:

Eleven commenters (#4, 8, 18, 23, 26, 28, 30, 31, 33, 34, 35) had concerns about Inventory listing. While ten of the commenters requested clarifications or modifications to EPA's proposed approach, one commenter (#31) felt that EPA should not finalize the Inventory portion of the proposal, but should hold public meetings to develop an appropriate Inventory nomenclature scheme for microorganisms.

Four commenters (#8, 18, 30, 35) asked that EPA clarify the type of taxonomic designation to be used for Inventory listing. Five commenters (#4, 8, 18, 30, 35) said that it was necessary for EPA to indicate how revisions to taxonomy would be accommodated on the Inventory. Two commenters (#34, 35) suggested that EPA consider Inventory listings for microorganisms to be inclusive of any taxa listed under historical or future revisions of the genera, as long as the microorganism lineage can be appropriately documented.

Three commenters (#28, 33, 35) indicated that EPA should clarify what is "new" under TSCA by limiting Inventory listing for a microorganism to the genus and species of the recipient microorganism and a description of the introduced genetic material. Four commenters (#28, 33, 34, 35) specifically opposed the proposal to use designations to the strain level.

Six commenters (#23, 26, 28, 33, 34, 35) encouraged EPA to adopt a policy that would not consider "new" under TSCA minor changes made during strain improvement of microorganisms already listed on the Inventory. Five commenters (#26, 28, 33, 34, 35) suggested that this could be accomplished by focussing on intergeneric expression of new phenotypic traits. One commenter (#23) suggested that EPA focus on traits of an organism likely to pose risk.

EPA response:

On the basis of two considerations, EPA believes it is prudent to defer a fuller development of Inventory listing for microorganisms. First, EPA recognizes that the method of listing on the Inventory is closely tied to the interpretation of what constitutes a new microorganism. Second, EPA agrees that Inventory listing for intergeneric microorganisms is more complex than listing for

most traditional chemicals. As indicated above in Unit II.D., EPA plans to address modifications and clarifications to its intergeneric interpretation in the future. Future modifications to the intergeneric interpretation could also affect how microorganisms are listed on the Inventory. A subcommittee of EPA's Biotechnology Science Advisory Committee (BSAC), which met on July 22, 1991, when questioned on EPA's proposed approach to Inventory listing for microorganisms, suggested that EPA continue on a case-by-case basis and gain additional experience before finalizing its requirements for Inventory listing. Pending further consideration, EPA will continue to use a case-by-case approach to Inventory listing for new microorganisms.

EPA believes that the preamble discussion of Inventory listing in the proposed rule (59 FR 45551-52 (September 1, 1994)), can serve as guidance to persons who are preparing MCAN submissions and need to address the microorganism identification requirements in § 725.12. Additionally, in Unit II.D. of this document, EPA has provided some clarification regarding use of taxonomy. As always, submitters may consult with EPA concerning clarification of their reporting obligations for specific microorganisms.

3.<u>"Grandfather" period</u>

<u>Comments</u>:

Three commenters (#25, 28, 33) requested that EPA provide a "grandfather" period by opening up the Inventory for one year after the final rule is published to allow products currently in commerce to be listed. One commenter (#39) requested that intergeneric products currently in commerce be automatically placed on the Inventory.

<u>EPA response</u>:

EPA disagrees with the commenters who believe that a "grandfather" period is necessary. Since the publication of the 1986 Policy Statement in June 1986, EPA has required PMN reporting for general commercial use of intergeneric microorganisms subject to TSCA. Although different scopes of oversight have been discussed in the intervening years, the Policy Statement has remained in effect all that time. Therefore, EPA believes that the public has had sufficient notice of its program and that intergeneric microorganisms currently in commerce and being used for TSCA purposes should have been reported to EPA.

4.<u>Microorganisms currently listed on the Inventory</u>

<u>Comments</u>:

Two commenters (#4, 24) discussed concerns about the 192 microorganisms currently listed on the Inventory by genus and species only. One commenter (#24) stated that there was no information about the phenotypic characteristics of these strains or about any introduced DNA. This commenter noted that "[w]ithout clarification of what specifically appears on the list, every

intergeneric strain would be subject to regulation by default." With regard to the Inventory, the other commenter (#4) stated "[p]resumably these are listed under species names available for biosafety regulators at [a] certain time period." This commenter gave specific examples of changes in taxonomic designation that have occurred for some of the microorganisms listed on the Inventory and suggested that EPA devise a method for keeping up with changes in taxonomy.

EPA response:

EPA wishes to clarify its position on microorganisms currently listed on the Inventory. These microorganisms can be divided into two groups: (1) those reported to the initial Inventory in the late 1970s, and (2) those listed after EPA's review of PMNs and receipt of Notices of Commencement to manufacture. EPA has no concerns about the Inventory status of the second group, because these microorganisms were all reported to EPA under the 1986 Policy Statement and therefore are intergeneric and are appropriately explicitly listed. The listings for these microorganisms include descriptive information to specifically identify them beyond the genus and species designations.

Such is not the case for the first group, the 192 microorganisms reported to the initial Inventory. As one commenter noted, these microorganisms are primarily listed by genus and species. EPA believes that most of these microorganisms are naturally occurring or have been modified by methods that do not involve the introduction of genetic material from an organism in another genus and thus in many cases would not need to be explicitly listed. To confirm this assumption, EPA requested that persons manufacturing or importing any of the 192 microorganisms inform EPA of their status during the public comment period for the proposed rule. No comments were received on the status of these microorganisms. EPA wishes to ensure that all microorganisms which are explicitly listed on the Inventory are intergeneric and are described in a consistent manner. Based on the absence of additional information, EPA has concluded that the 192 microorganisms are not intergeneric and, thus, are automatically on the Inventory under §725.8(b). EPA will remove the explicit listings from the Inventory in a separate action under the authority of TSCA § 8(b).

With regard to the concerns about changes in taxonomy, as discussed above, EPA will address its approach to Inventory listing in more detail when it develops modifications to its intergeneric interpretation. In the interim, EPA has indicated how it will address taxonomy issues in Unit II.D. of this document.

C.CONFIDENTIAL BUSINESS INFORMATION

EPA proposed to require upfront substantiation of confidential business information (CBI) claims in all submissions for general commercial uses of microorganisms: anyone submitting a MCAN, a TME, Tier I certification, or a Tier II exemption request would be required to substantiate CBI claims at the time of submission. With respect to upfront substantiation for TERAs, EPA

proposed two options and asked for public comments on both. Option 1 would require upfront substantiation of all CBI claims in TERAs. Option 2 would not require upfront substantiation of CBI claims in TERAs, but would only require CBI substantiation after EPA received a Freedom of Information (FOIA) request.

Comments:

EPA received 11 comments (#8, 12, 15, 18, 23, 24, 25, 28, 31, 33, 35) regarding confidential business information (CBI) claims for microorganism submissions. One commenter (#24) asked for additional clarification of EPA's CBI policy for microorganism submissions. Two commenters (#12, 15) supported EPA's proposal to require upfront substantiation of CBI claims for submissions for both research and general commercial use.

Eight commenters (#8, 18, 23, 25, 28, 31, 33, 35) opposed upfront substantiation of CBI claims in microorganism submissions. Three of these commenters (#8, 18, 25) specifically opposed upfront substantiation of CBI claims in submissions for R&D purposes only, indicating that the requirement was too burdensome for R&D because it was important to have proprietary protection for R&D activities. The other five commenters (#23, 28, 31, 33, 35) opposed upfront substantiation of CBI claims for both R&D and general commercial use submissions, arguing that EPA's approach to substantiation of CBI claims in microorganism submissions should not differ from EPA's approach to substantiation of CBI claims traditional chemical submissions.

EPA response:

In setting its requirements for CBI substantiation, EPA must balance the competing needs of opening the review of submissions for microorganisms to public scrutiny and participation while protecting legitimate CBI claims. EPA recognizes that industry believes that the requirement for upfront substantiation of CBI claims imposes a greater burden on R&D submitters than is necessary and that proprietary protection for R&D is essential. In the past several years, submitters of voluntary PMNs for field tests of new microorganisms have claimed very little, if any, CBI. These submitters have indicated to EPA that they are anxious to have an open process, so that the public will gain an understanding of their work and find the microbial products acceptable when they finally reach the market. Based on this experience and considering the comments received, EPA has decided not to require upfront substantiation of CBI claims in TERAs. However, if, in the future, EPA finds that CBI claims have increased in TERAs and that insufficient information is available to the public during the 60-day TERA review period, EPA may find it necessary to reconsider this decision. The regulatory text at § 725.94(a)(2) has been revised to read as follows:

(2) TERA requirements. Any person who submits a TERA, should strictly limit confidentiality claims to that information which is confidential and proprietary to the business. If any information in such a submission is claimed as confidential business information, the submitter must have available for each of those claims, and agree to furnish to EPA upon request, written answers to

the questions in paragraphs (d) and (e) of this section.

In the case of general commercial use submissions, EPA believes that the upfront substantiation requirement for CBI claims will impose little burden on submitters of MCANs, TMEs, Tier I certifications, and Tier II exemption requests. Because submitters of MCANs, TMEs, Tier I certifications, and Tier II exemption requests are ready to put their products on the market, they should be able to justify why it will continue to be necessary to keep certain information confidential. In addition, given the shorter review period for TMEs and Tier II exemption requests, sufficient information may not be made available to the public if upfront substantiation of CBI claims is not required.

D.TSCA SECTION 8(e)

In the proposal, EPA reminded persons manufacturing, importing, processing, or distributing in commerce any TSCA-covered microorganisms of the responsibility under TSCA § 8(e) to immediately report to EPA any information the person obtains which reasonably supports the conclusion that such microorganisms present a substantial risk of injury to health or the environment.

Comments:

EPA received four comments (#8, 25, 28, 33) regarding TSCA § 8(e) reporting requirements. All four commenters indicated that while the § 8(e) reporting requirements that were originally developed for chemicals could be applied to microorganisms, they believed that EPA should develop clear guidance specifically for microorganisms. Two commenters (#28, 33) asked that EPA clarify "that products not subject to § 5 notification are still subject to § 8(e) requirements."

EPA response:

TSCA § 8(e) requires all manufacturers, processors, and distributors of a chemical substance or mixture subject to TSCA to notify EPA immediately of any new information which reasonably supports the conclusion that such substance or mixture presents a substantial risk of injury to health or the environment. This requirement applies to all chemical substances and mixtures that are subject to TSCA, regardless of whether they are subject to TSCA § 5 notification. Therefore, all microorganisms and microbial mixtures that are subject to TSCA, including naturally occurring and intrageneric microorganisms, as well as intergeneric microorganisms are subject to TSCA § 8(e) reporting requirements. The requirements also extend to those involved in research and development activities with microorganisms and microbial mixtures subject to TSCA.

EPA does not agree with commenters who believe that specific guidance for § 8(e) reporting of microorganisms is necessary. While EPA recognizes the biotechnology industry's desire for

explicit guidance, given the broad nature of § 8(e) and the diverse types of studies and other information that can fall within the overall scope of the required reporting, it is unlikely that EPA would be able to provide fully explicit reporting requirements that would cover every hypothetical circumstance involving the diversity of microorganisms and uses potentially subject to TSCA.

Over the years, EPA has spent considerable effort to prepare and disseminate interpretive guidance for § 8(e) reporting for traditional chemicals. EPA believes that this guidance can be applied to microorganisms. While the specifics may be different for microorganisms, the decision-making process should be similar. In deciding whether information is "substantial risk" information, a person must consider (1) the seriousness of the adverse effect, and (2) the fact or probability of the effect's occurrence. In determining § 8(e) reportability, these two criteria should be weighted differently depending upon the seriousness of the effect or the extent of the exposure. The decision-making process for § 8(e) reportability should focus primarily on whether the hazard or exposure information offers reasonable support for a conclusion of substantial risk, but should not focus at all on whether the information is conclusive regarding risk. Determining whether reasonable support exists for "substantial risk" is not synonymous with the determination of an "unreasonable risk" as that term is used in § 5 of TSCA. Guidance regarding the § 8(e) reporting requirements may be obtained from the TSCA Assistance Information Service at (202) 554-1404; TTD (202) 554-0551; on line service modem (202) 554-5063.

E.ANTIBIOTIC RESISTANCE MARKERS

Although EPA only discussed the use of antibiotic resistance genes as markers as part of its proposal for exempting two specific microorganisms from TERA reporting (see Unit IV.F.), EPA also received comments addressing more generally the use of antibiotic resistance markers.

Comments:

EPA received 11 comments (#2, 5, 6, 8, 12, 14, 15, 16, 20, 24, 27) generally addressing the use of antibiotic resistance markers. One commenter (#8) supported the use of antibiotic resistance genes as selectable markers for microorganisms. Six commenters (#2, 6, 12, 14, 15, 20) indicated that antibiotic resistance genes should not be used with intergeneric microorganisms that would be released into the environment. One commenter (#12) indicated that EPA should also consider limiting the commercial use of microorganisms containing certain endogenous antibiotic resistance markers.

Three commenters (#5, 12, 27) specifically opposed the TERA exemptions for *Rhizobium* and *Bradyrhizobium* strains at § 725.239 that allowed the use of antibiotic resistance markers from any source. Three commenters (#12, 16, 27) more generally opposed exemptions for microorganisms containing antibiotic resistance markers.

Two commenters (#8, 24) suggested that EPA develop a list of antibiotic resistance markers that

should not be used. One commenter (#8) indicated that other types of markers should be exempt from TSCA as an incentive for their use. One commenter (#15) suggested that EPA convene another meeting of its BSAC to consider the issue of antibiotic resistance markers. Two commenters (#16, 27) indicated that there were other types of markers which were more acceptable than antibiotic resistance markers. One commenter (#12) suggested that EPA provide a list of other markers to use.

EPA response:

EPA did not discuss a general policy for addressing use of antibiotic resistance genes as markers as part of its proposed rule. Use of antibiotic resistance markers was only discussed as part of the exemption from TERA reporting proposed for certain modified strains of *Bradyrhizobium japonicum* and *Rhizobium meliloti*. EPA has responded to comments on the TERA exemption in Unit IV.F. above, and has revised the regulatory text at § 725.239 regarding the use of antibiotic resistance markers in *Bradyrhizobium japonicum* and *Rhizobium meliloti* in response to specific comments.

EPA recognizes that many factors affect the health and safety evaluation of use of antibiotic resistance markers. The use of antibiotic resistance markers is a complicated issue which has ramifications for products beyond the scope of TSCA. Because of the complexity of the issues, EPA will not develop a general policy on the use of antibiotic resistance markers, but will continue to evaluate their use in specific microorganisms on a case-by-case basis as submissions are received. EPA plans to pursue this issue in consultation with other Federal agencies who have an interest in this issue.

F.STATE COORDINATION

The proposed rule discussed EPA's procedures under the 1986 Policy Statement for coordinating reviews and sharing scientific information with appropriate State and local authorities. EPA proposed to require persons preparing TERA submissions for R&D activities involving release to the environment to provide evidence in the submission of having notified appropriate State authorities.

Comments:

EPA received five comments (#8, 18, 31, 33, 37) on its proposal for coordination with appropriate state authorities. One commenter (#37) supported EPA's proposed requirement for State coordination. Three commenters (#8, 18, 31) opposed the requirement. Two of the three commenters believed the requirement was a disincentive for R&D, but that if applied, it should only be applied to environmental R&D. The other commenter (#31) stated that the requirement should be deleted, because the terms "appropriate Federal and State authorities" are not defined

and "this requirement is impermissibly vague and ambiguous." One commenter (#33) expressed concern about adequate protection of CBI during such activities.

EPA response:

Based on comments as well as its experience, EPA has revised §§ 725.238(b)(3)(ii) and 725.255(e)(1)(vi) which required submitters to include evidence that State authorities have been notified in the TERA exemption certification and TERA submission, respectively. The provision has been revised to read, "If State and/or local authorities have been notified of the activity, evidence of notification." EPA's reasons for the change are discussed below.

EPA has developed comprehensive procedures to coordinate reviews of submissions and to share scientific information with appropriate State and local authorities to the fullest extent possible without violating TSCA CBI requirements. In all circumstances, EPA adheres strictly to its procedures for handling TSCA confidential business information (CBI). Because EPA maintains a public docket, nonconfidential versions of many documents must be prepared and EPA can use these nonconfidential documents to share information with interested parties, including State and local authorities.

Under EPA's current procedures for review of field tests under the 1986 Policy Statement, an EPA review coordinator contacts by telephone the appropriate regulatory agencies in the State(s) where the test will be conducted to inform them of the submission. If requested, a nonconfidential copy of the submission is mailed to the State contact. Nonconfidential reports, assessments and public comments added to the Public Docket are routinely made available to State personnel upon request. Comments and concerns raised by the State(s) are given careful attention during the review process. State personnel receive a copy of any document which addresses the conditions under which the field test can be performed.

Some states have requirements that State authorities be given copies of information that is sent to Federal authorities for field tests of certain designated microorganisms. Only one requires a separate review process. EPA believes that it is important to involve appropriate State and local authorities in the review of field tests, because these tests are occurring in their jurisdictions and because it is not possible for EPA personnel to visit every field test site. If any problems result from the field test, EPA would coordinate with State and local personnel. Therefore, EPA will continue to encourage submitters to advise state and local authorities of their field test plans, although this will not be a requirement. In cases where submitters do inform state and local authorities of their test plans, EPA believes that it is appropriate to require that submitters inform EPA of this notification as part of their TERA submissions and TERA exemption certifications.

G.REGULATORY DECISION

During the review of a submission for a microorganism, EPA may reach one of three decisions, based on a balancing of the risks and benefits presented by the microorganism: (1) The available

information does not indicate that the risks are unreasonable; (2) there is sufficient information to determine that the risks are unreasonable; or (3) there is insufficient information to make a reasoned evaluation of risk, and the substance may present an unreasonable risk or the microorganism will be produced in substantial quantities, and the microorganism may reasonably be anticipated to enter the environment, or there may be significant or substantial human or environmental exposure to the microorganism. These three potential decisions were discussed briefly in the proposal.

Comments:

EPA received three comments (#28, 33, 39) on its regulatory decision making. Two commenters (#28, 33) stated that:

EPA should establish in the regulation itself what standards they will use in determining what is a 'significant or substantial human or environmental exposure.' This will ensure that manufacturers address Agency concerns prior to submission and are not 'surprised' by an EPA decision of 'unreasonable risk.' For those submissions where there is insufficient information to determine that the risks will not be unreasonable, EPA should document in detail its concerns to the manufacturer. This will aid the manufacturer in reaching a decision as to whether to proceed with the planned commercialization.

A third commenter (#39) stated:

It is essential for effective management of commercial R&D that a company be able to make reasonably accurate predictions of whether or not a future chemical or microbial product will or will not be allowed by EPA. ... These predictions of regulatory risk cannot be made unless EPA clearly states the evaluation criteria that will be used to approve each application level. We therefore request that EPA clearly lay out the review criteria and risk evaluation procedures for each level. For example, EPA might wish to consider as a model the Structure Activity Relationship Criteria that EPA currently applies to assessment of new chemicals. ... we recommend that EPA conduct a separate rulemaking proceeding in order to establish such criteria.

EPA response:

As noted above, during the review of a submission, EPA may make one of the three regulatory decisions. Because this decision is made in a case-by-case analysis based on a balancing of the risks and benefits presented by the specific microorganism under review, EPA does not believe that it is possible *a priori* to develop "one size fits all" standards for this determination.

The term "unreasonable risk" is not defined in TSCA. However, TSCA § 6(c) lists considerations for determining whether a substance presents an unreasonable risk for purposes of promulgating regulations under TSCA § 6. The considerations include the effects of the substance on human

health and on the environment and the magnitude of exposure to the substance, the benefits of the substance for various uses, the availability of substitutes for such uses, and the reasonably ascertainable economic consequences of the potential regulatory action. A review of the legislative history of TSCA shows that the House Report (H.R. Rep. 94-1341, 94th Cong., 2d Sess, at 13-15, 32) states that the unreasonable risk standard cannot be defined in precise terms but, instead, requires exercise of judgment by the decision maker. The House Report describes the finding of unreasonable risk as involving a balancing of the probability that harm will occur, and the magnitude and severity (potential consequences) of that harm, against the effects (social and economic) of proposed action on society.

According to the House Report, these evaluations of harm often must be based on considerations of "scientific theories, projections of trends from currently available data, modeling using reasonable assumptions and extrapolations from limited data." (House Report, at 32). The unreasonable risk standard recognizes that, as a practical matter, all scientific evidence is uncertain to some degree and that EPA can consider such factors as the strength of the evidence on toxicity, the nature of the effects that may occur (e.g., death vs. reversible effects), and the likely numbers of individuals exposed and the levels of exposure. The House Report points out that the unreasonable risk standard is flexible enough to allow EPA to calibrate the stringency of a regulatory measure to the levels of risk and benefits. Therefore, if EPA determines that there is insufficient information to permit a reasoned evaluation of the health and environmental effects of a microorganism and either the microorganism may pose a risk to human health or the environment or the microorganism will be produced in substantial quantities, and the microorganism may reasonably be anticipated to enter the environment, or there may be significant or substantial human or environmental exposure to the microorganism, TSCA § 5(e) allows EPA to enter into a consent order with the submitter allowing the submitter to manufacture the microorganism under restricted conditions and requiring the collection of data to better characterize the microorganism. If EPA determines that there is a reasonable basis to conclude that a microorganism will present an unreasonable risk, TSCA § 5(f) allows EPA to issue an injunction to prohibit the manufacture, processing, or distribution in commerce of the microorganism.

Because EPA encourages prenotice consultations and keeps a public docket for each § 5 notice, EPA does not believe that manufacturers filing microorganism submissions should be "surprised" by decisions made under TSCA § 5. For most of the notices that EPA has reviewed for microorganisms used as intermediates to produce specialty chemicals such as enzymes, EPA has concluded that the available information does not indicate that the risks are unreasonable. Therefore, the submitters have been free to begin manufacture of the microorganisms after the expiration of the review period. In fact, many of these microorganisms have been proposed for the tiered exemption discussed above in Unit III.C. To date, EPA has not used TSCA § 5(f) to regulate microorganisms.

The greatest uncertainty has occurred with reviews of proposed field tests of new microorganisms. In these cases, where EPA did not identify significant concerns based on the

available data, including greenhouse or other contained studies, it decided to allow the field tests to proceed. When it was necessary, in order to develop the information EPA needed to reduce uncertainty about microorganism behavior such as survival and dissemination, and when this information could only be obtained from field testing, EPA has used its authority under TSCA § 5(e) to negotiate consent orders with submitters to allow limited field tests. Thus, in anticipation of possible commercialization, the 5(e) Consent Order has enabled the submitter to proceed with product development while providing EPA with additional data needed to reduce uncertainty. As questions were answered, EPA reduced the requirements it imposed on subsequent field tests.

In general, as EPA has gained additional experience reviewing both sequential field tests with microorganisms and commercial uses of fermentation microorganisms, it has been able to reduce requirements where uncertainty has been reduced. This has been true as EPA has worked with specific submitters and as EPA has tried to generalize that experience through the development of flexible exemptions which can be expanded. EPA expects to continue with this approach in the future.

H.ECONOMIC IMPACT

EPA prepared a Regulatory Impact Analysis (RIA) assessing the costs, benefits, and associated impacts of regulating new microorganisms under TSCA as set forth in the proposed rule. Based on the analysis presented in the RIA, EPA's preliminary findings were that the proposed rule should not adversely affect either innovation or international competitiveness in biotechnology. EPA requested comments on the economic impacts associated with the proposed rule and made the RIA available to the public as part of the rulemaking docket. EPA has revised the RIA to reflect changes made to the proposed rule. The RIA which supports the final rule is included in the rulemaking docket.

1.Impacts on innovation

Comments:

EPA received comments regarding the potential for the rule as proposed to hinder innovative research. One commenter asserts that "the rule will potentially enable only high value microorganisms to be commercialized," while "microorganisms of limited use, both temporally and spatially, are unlikely to be developed" (#8). Another echoed this position, stating that "only those experiments likely to lead to a high-value product will be conducted and only those institutions with sufficient capital will be able to compete" (#24). A more general statement made by an additional commenter expressed concern in connection with the potential impacts on R&D activity resulting from "early stage regulatory oversight," and included an example of potential impacts associated with "multiple regulatory jurisdictions" in the area of bioremediation (#33).

EPA response:

EPA is sensitive to the concerns expressed regarding the impacts of the rule on innovation and on the competitive structure of the industry. In its RIA, the Agency conducted an investigation into the potential impacts of the costs associated with reporting on product development costs and schedules (see RIA, App. F). This analysis demonstrated that a wide range of impacts could be realized, but that such impacts were highly dependent on project characteristics and review duration. In general, the results suggest that larger scale, higher return projects could indeed be less likely to experience substantial impacts; however, smaller scale projects modeled also exhibited financial viability when regulatory burdens were considered as part of the product development process. Importantly, review duration (termed "delays" in the analysis) played a significant role in the severity of impact sustained by any particular project.

Because smaller scale projects of limited use would most likely be exempt (e.g., organisms used exclusively for research purposes) or involve a relatively limited set of use and exposure scenarios, delays due to regulatory review would be expected to be non-existent or minimal; thus, the impacts noted above by commenters could be mitigated in many situations of the type described. EPA also emphasizes that a number of burden-reducing provisions have been included in its final rule: the TERA process, which streamlines field trials for R&D; the TERA exemption and tiered exemption provisions, which reduce data requirements in connection with more familiar microbiological products; and the exemption for laboratory research conducted in contained structures.

Though EPA requested data regarding impacts and product development issues in the preamble to the proposed rule (59 FR 45557 (September 1, 1994)), no such data were submitted. Neither were specific comments received pertaining to the analysis or cash-flow models presented in the RIA. In the absence of such comments, and in light of the flexibility incorporated into the final rule, the Agency has determined that innovative impacts of the rule should not hinder the industry from pursuing a full range of product applications.

With respect to the specific case of multiple regulatory jurisdictions in the area of bioremediation, it was not made clear by the commenter why these regulations promulgated under TSCA would limit the ability of firms to conduct performance testing necessary for remediation technologies, as required under RCRA and CERCLA. Assuming that such an application for an intergeneric microorganism subject to TSCA was under development, the detailed information necessary to establish the performance of the product would likely provide a good deal of the informational requirements of the TERA (e.g., see RIA, App. D, Table D-3; and § 725.255, "Information to be Included in the TERA"). Thus, while the reporting requirements of these rules could introduce some additional costs into the product development process, such incremental costs are likely to be a small component of overall development costs for this type of product, that is, a large portion of TSCA-related regulatory costs may be incurred in any event as part of the product development and CERCLA review process. Therefore, EPA does not believe, as a general rule, that TSCA oversight should add significantly to existing resource requirements necessary to develop bioremediation products. Unfortunately, data regarding such development costs, particularly costs attributable to the type of performance testing alluded to by the commenter,

were not provided.

2.Impacts on international competitiveness

Comments:

A number of commenters expressed concern regarding the potential for the rule to adversely affect the competitive position of U.S. biotechnology firms in world markets. For example, one commenter claimed that additional regulatory burdens would be likely to have a negative impact on research funding, resulting in an erosion of the competitive position of the U.S. (#24) while another recommended the regulations be evaluated in comparison to other developed countries having significant biotechnology development resources (#8).

EPA response:

EPA recognizes that regulatory initiatives may have implications for U.S. based firms facing competition in the global marketplace. In its RIA, the Agency presented a thorough discussion of regulatory initiatives in key industrialized nations and found that, overall, similarities among the nations' regulatory approaches were more striking than the differences (see RIA, Executive Summary). In light of this, and the fact that any incremental regulatory burdens imposed by the rule are estimated to be modest and associated with a minority of cases (that is, most regulated firms should find regulatory burdens somewhat reduced due to exemption provisions incorporated into the rule), EPA does not anticipate significant impacts on international competitiveness related to this action.

It should be noted that no data or evidence were submitted by commenters supporting the assertion that the rule could result in erosion of the competitive position of U.S. firms. Presumably, any adverse impacts associated with TSCA regulation of products of biotechnology would be evident under current regulatory policy, upon which the final rule is based. Because incremental impacts associated with this rule are expected to only marginally increase impacts realized under current policy, EPA must conclude that commenters' concerns are largely speculative in nature, and that prevailing conditions most likely also reflect post-promulgation circumstances.

3.Cost estimates

Comments:

One commenter (#24) stated their view that EPA had significantly underestimated the costs of compliance with the rules. The commenter claimed that the EPA review process was similar to the filing of a patent application, and that administrative costs would range from $20,000 to $45,000 per application.

EPA Response:

In its RIA which accompanied the proposed rule, EPA presented detailed tabular summaries ("spreadsheets") of the Agency's estimates of incremental costs associated with the preparation and filing of two proposed reporting vehicles: the TSCA Experimental Release Application (TERA) and the Microbial Commercial Activities Notification (MCAN) (see RIA, App. D). The cost ranges estimated for the TERA and MCAN were $5,330-$54,425 and $6,931-$32,772, respectively. Though not specifically noted in the comment, EPA assumes that these reporting vehicles were the items of primary interest to the commenter.

While the commenter's estimated range of $20,000 to $45,000 per application exceeds the Agency estimates considerably at the low-end of the EPA ranges, the commenter's claim that the Agency has "significantly underestimated" the costs of the rule is not supported by these figures. Clearly, the upper end of the EPA range is comparable to the range presented by the commenter. EPA recognizes that certain information required to be submitted with the TERA or MCAN would likely not be necessary when filing a patent application, such as information regarding monitoring, confinement, mitigation, and emergency termination procedures for a field experiment. However, EPA estimated that this information represents less than one-third of the overall TERA filing cost at the high-end, and that a major portion of the burden associated with compiling this type of information is necessary to ensure an effective experiment, regardless of whether the TERA is filed or not. Therefore, while it would be expected that costs incurred in connection with TSCA oversight could often exceed costs incurred for patent applications, it is not obvious that, based on the comparison suggested by the commenter, the Agency's estimates significantly understate such costs routinely.

Further, it is not known to what type of microorganism the cost estimate provided by the commenter applies; as shown by the cost spreadsheets in the RIA, costs to prepare for TSCA review are expected to vary significantly based on the complexity and uncertainty associated with a particular microbiological product. Without more specific information describing the circumstances surrounding the patent and the extent of any documentation prepared for the application (e.g., written description of design, drawings), it is difficult for the Agency to determine how best to integrate the commenter's statement into the Agency's analysis. For the reasons explained above, and absent any specific criticisms of the RIA methodology or data sources, EPA concludes that the cost estimates presented in the RIA provide a representative assessment of the economic impacts of the final rule.

VI. APPENDIX

A.PUBLIC COMMENTERS

Comments on the proposed TSCA biotechnology rule were received from the following public commenters who are listed by the number assigned to them in the public docket. With the exception of the letters which were solely requests for extension of the public comment period

(#1, 3, and 10) and one duplicate comment (#19), all other comments are referred to in this document by the number indicated below:

1.Genencor (extension request only)
2.Citizens for Accountable Genetic Engineering (CAGE)
3.DuPont (extension request only)
4.Alexander Mazanov
5.Science Mediation Service
6.Farm Verified Organic, Inc.
7.Association of American Medical Colleges (AAMC)
8.American Society for Microbiology (ASM)
9.Earlene K. Busch
10.Chemical Manufacturers Association (extension request only)
11.Cooley Godward Castro Huddleson & Tatum
12.Environmental Defense Fund (EDF)
13.Mycogen Corporation
14.Organic Foods Production Association of North America
15.Union of Concerned Scientists (UCS)
16.U.S. EPA Region II
17.Michael A. Cole, University of Illinois at Urbana Champaign
18.American Phytopathological Society (APS)
19.Farm Verified Organic, Inc. (duplicate of #6)
20.F.U.T.U.R.E. Organics, Inc.
21.United States Biochemical (USB)
22.Council on Governmental Relations (COGR)
23.D. Glass Associates, Inc.
24.Society for Industrial Microbiology (SIM)
25.Monsanto
26.Energy BioSystems Corporation (EBC)
27.Project to Label Gene-Altered Food
28.Genencor
29.University of Arizona, IBC
30.Minnesota Department of Agriculture
31.DuPont
32.Pioneer Hi-Bred International, Inc.
33.Biotechnology Industry Organization (BIO)
34.Enzyme Technical Association (ETA)
35.Novo Nordisk
36.American Seed Trade Association, Inc. (ASTA)
37.Wisconsin Dept. of Agriculture, Trade & Consumer Protection
38.ChromaXome
39.Life Technologies (LTI)
40.Asgrow

B.REFERENCES

The following books, articles, reports and telephone logs were used in preparing this document and were cited in this document by the number indicated below.

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